Exhibit A

Postprandial Plasma Glucose, Insulin, Glucagon and Triglyceride Responses to a Standard Diet in Normal Subjects

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Summary. Postprandial plasma glucose, insulin and triglyceride responses were determined in 12 normal subjects (7 male and 5 female) fed a standard diet composed of typical American foods; the three meals were identical for each subject. A significant postprandial rise in glucose and insulin was observed. They were closely related temporally in the early postabsorptive period. However, in the late post-absorptive phase insulin decline was generally slower than the glucose decline. A considerable difference in the glucose and insulin response was observed between males and females. Fasting plasma glucose and insulin concentrations were lower in the women. Following each meal the peak plasma glucose was lower in the women, but the difference was significant only following breakfast ($p < 0.02$). The area under the glucose curve following breakfast was also lower ($p < 0.01$) in the women. In the men the maximal postprandial glucose concentration and the postprandial glucose area remained stable throughout the day, but there was an increase in peak insulin concentration and insulin area after dinner. In contrast, in the women the maximal postprandial glucose concentration and the postprandial glucose area increased throughout the day, but the peak insulin concentration and insulin area did not change. Plasma triglycerides increased with breakfast and remained elevated throughout the day. Both fasting and postprandial mean triglycerides were higher in the men, but this did not reach statistical significance. The circulating pancreatic glucagon concentration, determined in 4 subjects, was unaffected by meals and remained stable throughout the day.

Key words: Glucose, insulin, triglycerides, glucagon, postprandial changes, sex-related differences.

Traditionally stimulation of insulin secretion has been accomplished by using individual nutrients such as glucose or amino acids in large and unphysiological amounts, or by administration of hormones such as glucagon or by administration of pharmacological agents such as tolbutamide. Recently there has been an increasing interest in analysis of B-cell function in physiological states approximating usual daily living conditions. This information should be useful in formulating sound principles for "diabetic diets" and for development of a more physiological insulin regimen.

There have been few studies concerned with the blood glucose and/or plasma insulin response to ordinary meals in human subjects. In 1946, Sindoni [1] reported that the average blood glucose level of 20 normal persons followed for a 4 hour period after a regular breakfast was little changed when compared with the fasting blood glucose. This lack of change in blood glucose was surprising and prompted us to study the plasma glucose and insulin concentrations over an extended period of time in normal human subjects eating three meals a day. Malherbe et al [2] also studied postprandial variations in blood glucose and plasma insulin in normal human subjects. They reported distinct peaks in blood glucose and plasma insulin concentration following each of three standard meals. While our study was in progress, other investigators [3–7] have reported plasma glucose and insulin concentrations following meals but the experimental design was different from that in the present study. In none of the previous studies have the results in men and women been examined independently. In addition we have studied plasma glucagon and triglyceride concentrations following standardized meals.

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Material and Methods

a. Subjects and Experimental Conditions

Twelve healthy, human volunteers (seven male and five female) were studied in a Metabolic Unit. One female subject was studied on two separate occasions 20 months apart. All subjects were Caucasian and were within 10% of ideal body weight using the Metropolitan Life Tables [8]. Mean age, weight and height for the males were 23 years (range 19–26), 169 lbs. (range 130–200), and 71 inches (range 69–74) respectively; those for the females were 24 years (range 19–32), 132 lbs. (range 114–145) and 66 inches (range 62–68) respectively. They had no known disease or disability and denied taking medication for four months prior to the study. There was no family history of diabetes mellitus. The following laboratory tests were within normal limits: Serum thyroxine, T_3 resin uptake, thyroid stimulating hormone, creatinine, urea nitrogen, uric acid, total proteins, albumin, bilirubin, lactic dehydrogenase, glutamic-oxaloacetic acid transaminase, alkaline phosphatase, cholesterol, triglycerides, calcium and phosphorus. All had a normal standard glucose tolerance test [9].

All participants signed an informed consent designed according to the Helsinki agreement on human experimentation and the study was approved by the hospital committee on human studies. The subjects were given a diet consisting of at least 300 g of carbohydrate per day for three days prior to testing. For the study on the fourth day they were given a diet composed of typical American foods (toast with margarine, bacon, ground beef patty, fruit, sweetened gelatin with whipped cream, cola-type carbonated beverage and coffee), and calculated to contain average proportions of protein, carbohydrate and fat. The total calories administered were based on the recommendations of the National Academy of Sciences [10] considering the differences in body weight, surface area, metabolic rates and energy requirements between males and females. Composition of the diet is shown in Table 1. The proportion of carbohydrates administered as simple sugars (monosaccharides and disaccharides) to the males and females was 61.3 and 61 percent of the total carbohydrates respectively. The remainder was given as starch. Diets used in other similar studies are shown for comparison. The total calories, protein, carbohydrate and fat were administered as three identical meals, i. e. breakfast, lunch and dinner were identical for each subject. They were served at 8 : 30 AM, 12 : 30 PM and 4 : 30 PM. Subjects were permitted to take their usual time to conjects were permitted to take their usual time to consume the meals. This ranged from 20 to 30 min. No other food or drinks were allowed except water ad libitum. Smoking was prohibited. The subjects ambulant during the study; they stayed in a reading, talking, playing cards or chess, or lister a radio, except for short walks on the same floor

Between 7 : 30 and 8 : 00 AM after an ove fast of 8–10 hrs, an indwelling catheter was in into an antecubital vein. This was kept opei syringe containing 100 units of heparin attachec catheter and 0.5 ml blood aspirated into the s This technique and the rapid frequency of bloo pling helped to keep the catheter open. Hepa not injected directly into the vein. Five heparinized venous blood was collected, be with two fasting samples at 8 : 15 and 8 : 30 A at 2, 5, 10, 20, 30, 40, 50 and 60 min after the ning of each meal for the first hour, every 15 the second hour and every 30 min for the thi In some subjects the 30 min sampling seque extended through the fourth hour. The blood were centrifuged immediately at 4°C, plasn rated and stored at −90°C until analysis for immunoreactive insulin (IRI) and triglycerid ing samples for glucagon and triglycerides w lected at 8 : 15 and 8 : 30 AM and then at 30, 180 and 240 (glucagon only) min after each m ml of blood for glucagon was collected in chil tubes containing 6 mg of Na_2EDTA and trasylol. Samples were centrifuged at 4°C plasma was divided into three equal aliquoi then frozen immediately under dry ice and −90°C until analysis.

b. Assays

Plasma glucose was determined in duplicate cose oxidase method using a Beckmar analyzer (Beckman Instruments, Inc., California). Plasma immunoreactive in measured in duplicate by a standard radioir say method [11] using insulin antibodies si Pharmacia Laboratories, Inc., Piscataway, ma glucagon was measured by radioimr [12], using antiserum 30-K, supplied by Unger; this antiserum is highly specific for glucagon but apparently does cross-react v inhibitory polypeptide [13]. Glucagon stan done in triplicate and the unknowns in dupl ma triglycerides were measured in duplica automated fluorometric method [14].

Coefficient of variation for plasma gl was 1.5% for samples in the range of 60–2 ml. Coefficient of variation for plasma IRI for samples in the range of 3–105 µU/ml. glucagon the coefficient of variation wa

samples in the range of 35–500 pg/ml and for tri-glycerides, 2.1% in the range of 50–300 mg/100 ml.

Statistical analysis of the results was done using the student "t" test for paired or unpaired variates as appropriate. The criterion of significance is a p value of < 0.05.

Results

a. Males

The mean plasma glucose concentration (Fig. 1) increased from a fasting level of 95 mg/100 ml to a peak value of 143 mg/100 ml at 40 min following breakfast. The peak glucose concentration after lunch and dinner was essentially the same as after breakfast. Plasma glucose concentration returned to the baseline value at 180 min after breakfast, but this was prolonged to 210 min after lunch and 240 min after dinner. A smaller second peak in glucose concentration was noted 60 to 120 min after each meal.

The increases in plasma insulin in general paralleled very closely those of the plasma glucose in the early post-absorptive period (Fig. 1). However, in the late post-absorptive phase insulin decline was generally slower than the glucose decline. A secondary peak in plasma insulin concentration similar to that for plasma glucose was evident in the late postprandial period after each meal. The concentration of plasma insulin increased from a fasting level of 25 µU/ml to 90 µU/ml at 50 min following breakfast. Following lunch and dinner the plasma insulin concentration rose to 92 µU/ml in 30 min and 107 µU/ml in 40 min

respectively. Plasma insulin concentration returned to baseline in 240 min following breakfast, but did not do so by 240 min following lunch and dinner.

b. Females

Following breakfast the mean plasma glucose concentration (Fig. 2) increased from a fasting value of 81 mg/100 ml to a peak value of 109 mg/100 ml in 30 min, then decreased to below the baseline and remained low until the next meal. The lowest mean plasma glucose concentration reached after breakfast was 63 mg/100 ml. It represented a fall of 46 mg/100 ml from the peak glucose concentration. This was not associated with any symptoms or signs of hypoglycemia. Five out of the six studies in females revealed a similar pattern following breakfast.

The peak plasma glucose concentrations following lunch and dinner were 118 and 127 mg/100 ml and occurred at 30 and 40 min respectively. The time taken for the plasma glucose concentration to return to the baseline was approximately 225 min after breakfast and 240 min after lunch, whereas, following dinner it had not returned to the baseline by 240 min.

Plasma insulin increased from a fasting value of 14 µU/ml to a peak value of 90 µU/ml at 50 min following breakfast. The peak plasma insulin concentrations after lunch and dinner were essentially the same as those seen after breakfast. Plasma insulin concentration returned to baseline 240 min following breakfast, but had not done so by 240 min following lunch and dinner.

In one female subject the study was prolonged until 12 midnight. Following dinner, plasma glucose

Table 1. Diet analysis

	Malherbe et al [2]	Hansen & Johansen [3]	Schlierf & Raetzer [6]	Genuth [7]	Present study	
					Males	Females
Total:						
Protein (g)	90	46	112	90	65	55
Fat (g)	75	73	94	81	160	113
Carbohydrate (g)	300	268	261	180	320	210
Alcohol (g)	–	48	–	–	–	–
Calories	2235	2249	2400	1800	3129	2089
% of Calories:						
Protein	16	8	19	20	8	10
Fat	30	30	36	40	47	48
Carbohydrate	54	48	43	40	45	42
Alcohol	–	14	–	–	–	–
% of Calories:						
Breakfast						
Lunch	33	20	33	33	33	33
Dinner	33	23	33	33	33	33
Snacks	33	36	33	33	33	33
	–	21	–	–	–	–

returned to the baseline in 75 min. Between 7 and 12 PM plasma glucose values fluctuated little with a maximum depression below the baseline not exceeding 6 mg/100 ml during this interval. In the same subject the highest plasma insulin value of 101 μU/ml was seen 40 min following dinner, then declined sharply to a level of 21 μU/ml at 60 min but did not return to baseline for 270 min. Thereafter insulin levels fluctuated modestly until midnight (data not shown).

Table 2 shows mean plasma glucose and insulin peaks computed from the indivudual values irrespective of when these occurred. For males the highest plasma glucose concentration following each meal was similar. For females the peak glucose concentrations following both lunch and dinner were statistically greater than after breakfast ($p < 0.05$ and < 0.02

respectively). The fasting glucose concentration shown in the table) and the highest plasma glu concentration following meals were both lowe females than in males. The difference in the peak: females than in males. The difference in the peak: significant ($p < 0.02$) after breakfast, but it di reach statistical significance for the other meals 0.1).

There was a gradual increase in the highest prandial insulin concentration in males as th progressed; however, these differences wer statistically significant. The postprandial peak i concentration was nearly identical for all three in the females.

Table 2 also lists areas under the glucos insulin concentration curves as determin planimetry and expressed as concentration pe
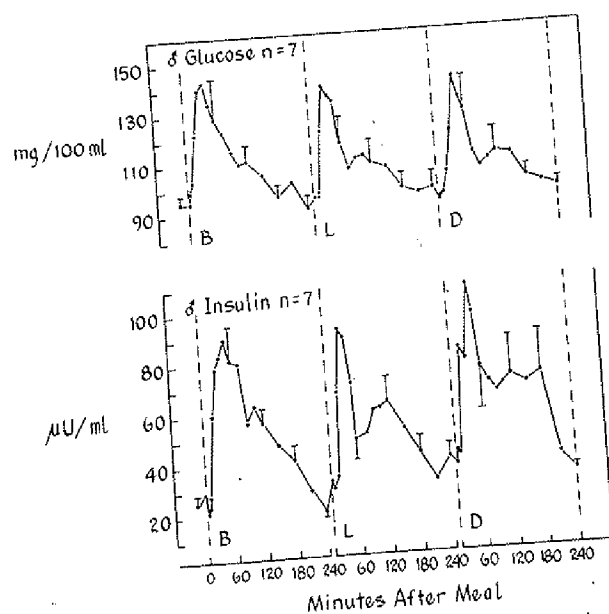

Fig. 1. Postprandial plasma glucose and insulin responses in 7 normal males. Dashed vertical line indicates the beginning of a meal. B (Breakfast), L (Lunch) and D (Dinner). Values are mean ± SEM
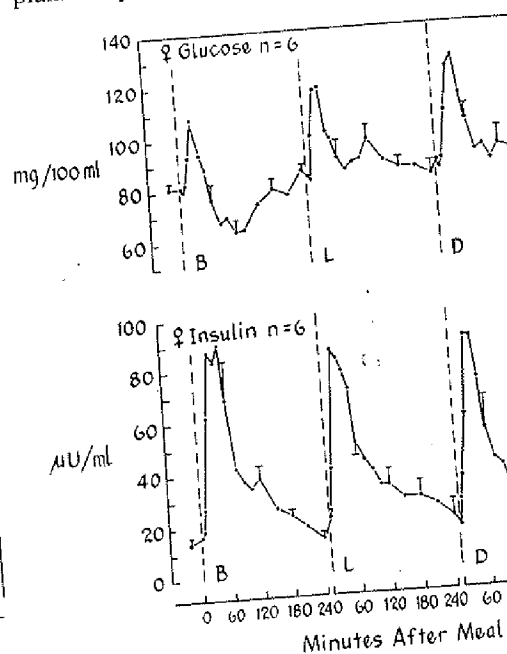
Fig. 2. Postprandial plasma glucose and insulin r normal females (6 studies). Dashed vertical line indic ning of a meal. B (Breakfast), L (Lunch) and D (D are mean ± SEM

Table 2. Highest concentrations and areas of glucose and insulin curves postprandially

	Peak glucose mg/100 ml		Peak insulin μU/ml		Glucose area mg/100 ml/min		Insulin area μU/ml/min	
	Males	Females	Males	Females	Males	Females	Males	Females
Breakfast	155 ± 13	111 ± 2[a]	99 ± 12	100 ± 13	1.4 ± 0.3	−0.2 ± 0.3[a]	2.9 ± 0.5	2.2 ± 0.3
Lunch	146 ± 8	125 ± 5[b]	103 ± 9	94 ± 10	1.2 ± 0.4	1.1 ± 0.4[b]	2.8 ± 0.3	2.0 ± 0.3
Dinner	145 ± 9	130 ± 6[b]	126 ± 16	102 ± 14	1.1 ± 0.2	1.2 ± 0.4[b]	3.7 ± 0.7	2.12 ± 0.4

Mean ± SEM
Males n = 7, Females n = 6
[a] Males versus females $p < 0.02$ or less
[b] Significantly different than breakfast $p < 0.05$ or less

ute. For males the area under the glucose curve was essentially the same after each meal; the area under the insulin curve following dinner was greater than that following breakfast or lunch, just as the peak insulin was higher, but again it was not statistically significant (p < 0.2).

For the females, the areas under the glucose curve following both lunch and dinner were significantly greater than after breakfast (p < 0.02 and < 0.01 respectively). However, the areas under the insulin curves remained unchanged after each meal.

The area under the glucose curve following breakfast was below the baseline in females and significantly different (p < 0.01) from that of the males. The total area under the plasma insulin curves following meals was greater in males, but this difference did not reach statistical significance (p < 0.1).

A quantitative estimate of insulin delivery to the peripheral circulation (excluding insulin removed by the liver on the first pass) was calculated from the integrated plasma insulin areas and the metabolic clearance data reported for normal persons [15, 16]. It was assumed that the insulin concentration remains low and rather constant during the night [2, 3, 5, 17] and that the metabolic clearance is constant [15–17].

For males it was 64 ± 7.6 (SEM) units and for females 47 ± 6.0 units daily. For all subjects it was 58 ± 5.0 units. These results are higher than those reported by Genuth (31 units) and calculated by him [7] from the data of Hansen and Johansen (26 units) [3]. The lower values can be attributed to differences in diet and the relative infrequency of sampling in these studies. The latter would result in an underestimation of insulin areas. A difference in insulin immunoassay method used also could contribute to the discrepancy.

Fig. 3 shows the plasma glucagon concentration in four subjects (2 males and 2 females). The mean basal level was 188 pg/ml and there was very little change throughout the duration of the study. Plasma glucagon concentrations were determined up to midnight in one subject and again there was little change. A significant alteration in plasma insulin/glucagon (I/G) ratio was present postprandially and this simply reflected the altered plasma insulin levels.

Plasma triglycerides (Fig. 4) increased after breakfast and remained elevated for the duration of the study in both males and females. There was a suggestion of a rise after both lunch and dinner but these were not significant. Both fasting and postprandial mean triglycerides were higher in males.

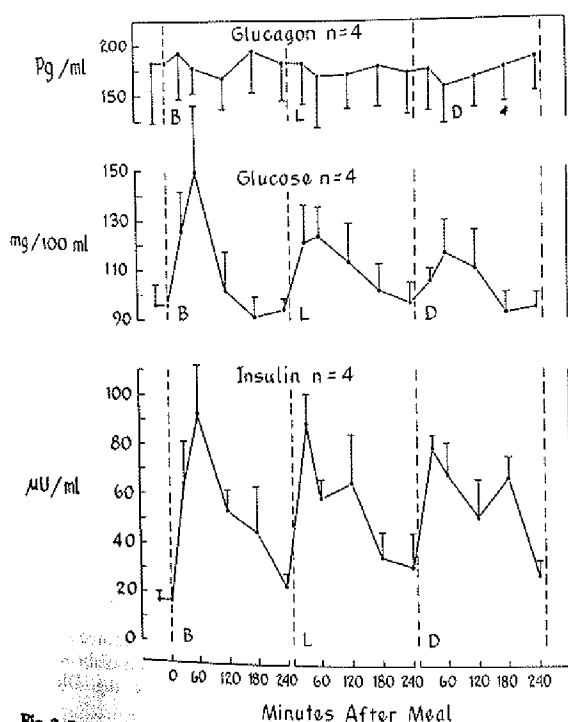Fig. 3. Postprandial plasma glucagon, glucose and insulin responses in 4 normal subjects (2 males and 2 females). Dashed vertical line indicates beginning of a meal. B (Breakfast), L (Lunch) and D (Dinner). Values are mean ± SEM
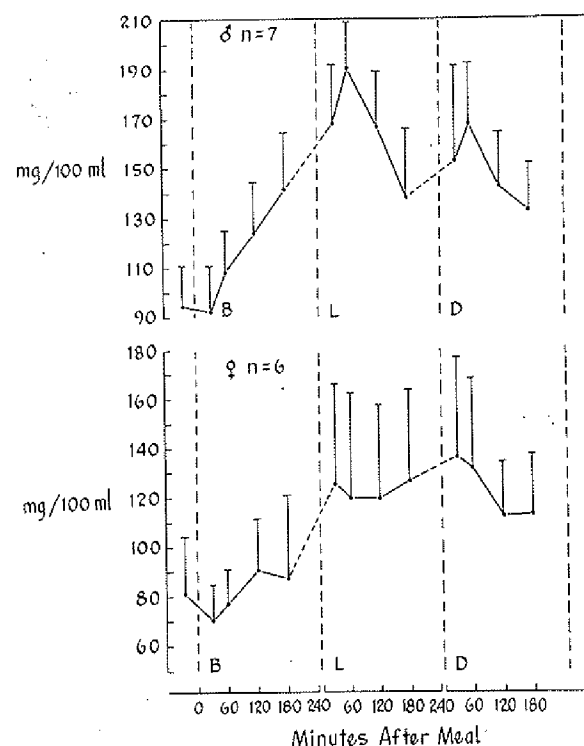

Fig. 4. Postprandial plasma triglyceride responses in 7 males and 5 females (6 studies). Dashed vertical line indicates beginning of a meal. B (Breakfast), L (Lunch) and D (Dinner). Values are mean ± SEM

Discussion

The previously reported studies [2–7] of circulating glucose and insulin concentrations following meals have all differed from the present study in regard to meal composition, timing of meals or timing and frequency of blood sampling, making comparison somewhat difficult. However, as in the present study, a significant postprandial rise in plasma glucose and insulin was observed and these were closely related temporally. In the present study there was also a delayed postprandial return of plasma glucose and insulin concentrations to baseline values after lunch and dinner. This appears to be present in other studies, [2, 3, 6, 7] and probably is due to a relative impairment in glucose tolerance late in the day [18].

The rate of rise in glucose and insulin immediately following the beginning of a meal is very rapid and the rise in insulin is proportional to the rise in glucose. Thus it is unlikely that "gut factors"[19] are significantly amplifying the glucose signal at the B-cell to a greater extent at this time than later in the postprandial period. Also a rise in insulin concentration does not occur prior to ingestion of food, even though in some cases, the individuals were not allowed to eat the food placed before them for several minutes. This suggests lack of a "cephalic" phase of insulin secretion mediated through the vagus nerve [20]. In the late postprandial period insulin concentrations were proportionally higher than the glucose values. Review of previously published studies indicates a similar dissociation although it was not commented on by the authors [2, 3, 6, 7]. The reason for this dissociation is uncertain. It is possible that factors other than glucose, such as amino acids or "gut factors", are stimulating insulin release at this time. Alternatively it could be due to a lag in response of the B-cells to a declining glucose concentration.

In previous studies males and females were not studied independently. Only Molnar *et al.*, [5] Schlierf and Raetzer [6] and Genuth [7] studied both males and females, but the data were not reported separately. In the present study a considerable difference in glucose and insulin responses was noted between males and females.

The reason for the sex-related differences in glucose and insulin responses after meals is unknown. Estrogen appears to enhance the rate of glucose removal [21] and a sex-related difference in plasma growth hormone level has been described [22, 23]. A difference in muscle mass relative to adipose tissue mass also could play a role. We have previously noted a sex-related difference in muscle glycogen synthase activation 30 min following glucose or insulin administration, [24] but not at a 60 min time period

(unpublished data). Interestingly Merimee and Tyson [25] have reported considerably lower plasma glucose concentrations in females during a 72 h fast. The mean glucose concentration also was lower in the females in the present study.

The circulating pancreatic glucagon concentration was unaffected by meals and remained stable throughout the day (Fig. 3). Hansen and Johansen [3] using an antibody which does not distinguish between gut and pancreatic glucagon have also reported little change in serum glucagon during a 24-hour period in three subjects fed a standard diet. Ingestion of a mixture of amino acids or a protein meal results in a rise in serum glucagon [26, 27] and ingestion of large amounts of glucose lowers the plasma glucagon concentration [28]. The lack of postprandial change in glucagon concentration in the present study possibly reflects the opposing influence of proteins and carbohydrates on pancreatic glucagon secretion. In normal individuals fed a high protein diet a rise in plasma glucagon occurs and it continues to increase throughout the day (unpublished observations).

Schlierf and Raetzer [6] have reported peaks in plasma triglyceride concentrations 5–6 hrs following a standard meal, resulting in approximate doubling of fasting triglyceride values, as was observed in the present study. In fat loading experiments triglyceride values have been reported to be higher in males than in females [29]. In the present study the mean triglyceride concentration was higher in males, both fasting and after meals, although none of these differences were significant due to the great variability found. In both sexes there was a gradual rise throughout the day.

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AND|[NOT_IDENT]

EFFECT OF ORAL GLUCOSE LOADING ON PLASMA INSULIN,
POTASSIUM, RENIN AND ALDOSTERONE IN NORMAL
SUBJECTS AND PATIENTS WITH PRIMARY
HYPERALDOSTERONISM

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ABSTRACT

The effects of standard oral glucose loading (100 g)
on plasma aldosterone and some regulatory factors were
assessed in patients with primary hyperaldosteronism and
normal subjects. Following overnight fast, mean plasma
glucose was identical (10 patients and normal subjects
approximately matched per age and sex); plasma insulin,
potassium and renin levels were lower and plasma aldo-
sterone higher in the patients. Glucose loading signi-
ficantly increased plasma glucose and insulin concen-
trations and decreased plasma potassium and aldosterone
levels in both groups; plasma renin activity was sig-
nificantly increased only in normal subjects. The in-
creases in plasma insulin and the decreases in plasma
potassium or aldosterone tended to be blunted in primary
hyperaldosteronism.
Relationships among glucose-induced changes in
plasma aldosterone and other factors were assessed by
multiple regression analysis in these patients and normal
subjects as well as an additional group of 21 normal sub-
jects; in the latter, plasma cortisol was also measured
and found to decrease significantly after glucose loading.
Changes in plasma aldosterone correlated ($P < 0.025$) more
closely with those in plasma potassium in the patients
and with variations in plasma renin activity in the
normal subjects. These findings suggest that complex me-
tabolic changes occur following glucose ingestion which

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are capable of modifying aldosterone secretion in normal
subjects and primary hyperaldosteronism. The aldosterone-
inhibitory effect of glucose tends to be blunted in the
latter disorder. This could be related at least in part
to an impaired insulin response in primary hyper-
aldosteronism.

INTRODUCTION

Aldosterone metabolism and potassium homeostasis
are closely interrelated. Increases in dietary potassium
intake or plasma potassium cause enhanced adrenal al-
dosterone secretion (5,14,21). On the other hand, aldo-
sterone contributes to the homeostasis of extracellular
potassium mainly by modulation of renal potassium ex-
cretion (17). Insulin also shows a similar reciprocal
interaction with this ion; its secretion from the pan-
creatic islet cells is stimulated by potassium (11,18)
while insulin is an important control factor of extra-
cellular potassium, since it facilitates the transfer
of this ion into the intracellular space (20,26). In
contrast to these stimulatory effects, potassium has an
inhibitory influence on renin release (32), and this in
turn modulates potassium metabolism by regulation of
plasma aldosterone levels (22). The interactions between
these factors cannot only be demonstrated under experi-
mental conditions, but occur also under physiological
conditions. In normal man, the increase in plasma insu-
lin following glucose ingestion is associated with a
slight decrease in plasma potassium, marked suppression
of aldosterone secretion, and mild renin stimulation
(1,21). Moreover, reciprocal circadian rythms of plasma
glucose and insulin on one hand, and plasma potassium
and aldosterone levels on the other hand, have been ob-
served (12).

In primary hyperaldosteronism, excess aldosterone
secretion from an adrenal adenoma induces sodium re-
tention, potassium depletion and hypertension (6). Aldo-
sterone release in such patients, though inappropriate,
may not be completely autonomous. The stimulatory in-
fluence of angiotensin II on the adrenal cortex was found
to be blunted (15,35). However, aldosterone was sti-
mulated acutely by corticotropin (4,16) or chroni-
cally by high potassium intake (16,23). Whether and to
what extent a mild reduction in plasma potassium may
acutely modify plasma aldosterone and renin levels in
primary hyperaldosteronism has not been studied. The
plasma potassium concentration is constantly modu-
lated by a variety of environmental factors, among
those carbohydrate intake (1,12,21). The present study
was undertaken to investigate the influence of a stand-
ard oral glucose load on plasma potassium, insulin, re-
nin and aldosterone levels and their interrelations in
patients with primary hyperaldosteronism as compared
with normal subjects.

SUBJECTS AND METHODS

Study A

Ten normal subjects (normal group A) and ten patients
with primary hyperaldosteronism were studied. The normal
subjects included five females and five males, ranging
in age from 22 to 61 years (mean 36 ± 4 ($\pm$SEM) years);
they were normal healthy volunteers with normal fasting
plasma glucose and creatinine levels and a blood pressure
consistently below 140/90 mm Hg. The patients included
eight females and two males, ranging in age from 19 to
58 years (mean 40 ± 3 years). The diagnosis of primary

hyperaldosteronism was based on the demonstration of an adrenal lesion by computer axial tomography and adrenal venography, of a gradient in the adrenal vein blood levels of aldosterone and, in six cases, by subsequent surgical removal of an adrenal adenoma. Plasma urea or creatinine levels were within the normal range in nine patients, plasma urea was slightly increased (9.2 mmol/liter) in one case. Where given previously, antihypertensive drugs, diuretics and potassium supplements were withdrawn at least two weeks prior to the test. Normal subjects and three of the patients were studied in Berne, seven patients were studied in Glasgow; all studies were supervised by CBP. Normal subjects and the three patients studied in Berne were instructed in a diet without very salty food and without adding salt to their food, starting at least five days prior to the test. Under these dietary conditions, mean urinary sodium excretion was 139 ± 6 mmol 24 hr^{-1} in our normal control population (2). In the three patients urinary sodium excretion in the 24 hr preceding the test ranged from 134 to 148 mmol 24 hr^{-1}. The seven patients studied in Glasgow were maintained on a diet with fixed sodium intake in the range 145-155 mmol 24 hr^{-1}, starting four to five days prior to the test (2).

Following an overnight fast, basal levels of blood pressure, plasma glucose, insulin, potassium, renin activity and aldosterone were measured following one hour of recumbency, blood samples being obtained through an indwelling intravenous cannula inserted 60 minutes previously. A glucose load of 100 g glucose in water was then administered, and the plasma concentrations of glucose, insulin, potassium, renin activity and aldosterone were determined 30, 60, 120 and 180 minutes thereafter while the subjects remained in the supine position.

Study B

To extend the findings in normal subjects, a second group (normal group B) of 21 healthy males (ranging in age from 22 to 47 years; mean 25 ± 1 years) was studied. Twenty-four-hr urinary sodium and potassium excretion averaged 134 ± 12 and 52 ± 7 mmol 24 hr, respectively. In addition to the investigations performed in group A (see above), these normal subjects had a further blood sampling at 15 minutes after the glucose load and measurements of plasma cortisol in all samples.

Study C

For comparison with the patients with primary hyperaldosteronism, an additional group of 16 subjects with secondary hyperaldosteronism was studied. Secondary hyperaldosteronism was induced by administration of the diuretic chlorthalidone (50 mg daily during the first two weeks and 100 mg daily during the second two weeks) in five normal (two females and three males) and 11 borderline hypertensive (22) subjects (four females and seven males). Age ranged from 21 to 60 years (mean 33 ± 4 years). Secondary hypertension in this group was excluded by the usual tests. Fasting plasma glucose and creatinine levels were normal in all subjects. To further stimulate the basal plasma aldosterone levels, the glucose loading test was performed in the sitting position. Basal blood samples were drawn following one hour of equilibration and 60 and 180 minutes following ingestion of 100 mg glucose in water for determination of plasma glucose, insulin, potassium and aldosterone levels.

Analytical Procedures

Plasma glucose was measured by the hexokinase method, plasma potassium by flame photometer, plasma insulin by

double isotope antibody assay (19), plasma renin activi-
ty (27) and aldosterone (13,30) by radioimmunoassay,
cortisol by competitive protein binding technique (31).

For t-test or regression analysis, the natural lo-
garithm transformation of plasma renin activity and al-
dosterone values was used. Differences between mean
values were tested by paired or unpaired two-tailed
t-test. Whenever correlations were calculated using a
series of consecutive measurements for each patient,
the mathematical integration over time of these serial
values was used.

RESULTS

Study A

On the day of the study, supine blood pressure
averaged 114/73 $\pm$ 9/4 mm Hg in patients with primary
hyperaldosteronism. Fasting plasma glucose was on aver-
age identical in both groups (90 $\pm$ 3 mg/dl). However,
compared to normal subjects, patients had lower levels
of insulin (18.7 $\pm$ 1.9 versus 10.6 $\pm$ 3.4 µE/ml; P<0.01),
potassium (4.14 $\pm$ 0.14 versus 3.26 $\pm$ 0.19 mmol/liter;
P<0.005) and plasma renin activity (1.54 $\pm$ 0.3 versus
0.53 $\pm$ 0.12 ng/ml/hr; P<0.01); while plasma aldosterone
concentrations were increased (2.8 $\pm$ 0.4 versus 30.2 $\pm$ 8.0
ng/dl; P<0.005).

Glucose ingestion induced significant increases in
plasma glucose or insulin and decreases in plasma potas-
sium or aldosterone levels in both normal subjects and
patients with primary hyperaldosteronism (Figures 1 and 2).
Maximal levels of plasma glucose and insulin were noted
30 minutes following glucose loading in normal subjects
(141 $\pm$ 10 mg/dl and 120 $\pm$ 13 uE/ml, respectively), but

Effects of Oral Glucose Loading on Plasma Glucose and Insulin in
Normal Subjects and Primary Hyperaldosteronism (Mean ± SEM)
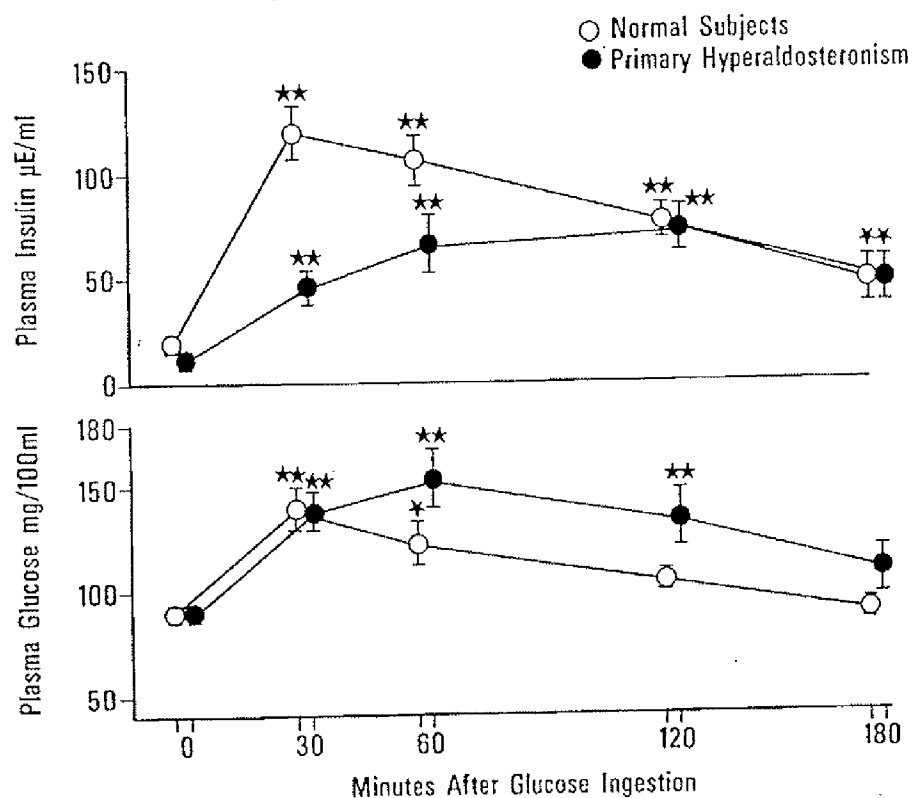
Figure 1

Symbols and bars indicate Mean ± SEM
* = P < 0.05; ** = P < 0.01 vs. corresponding basal value

somewhat later, at 60 to 90 minutes in the patients
(153 ± 14 mg/dl and 72.3 ± 9.5 µE/ml). Plasma insulin le-
vels at 30 and 60 minutes after glucose ingestion remained
lower (P < 0.05) in the patients than in normal controls.
Plasma potassium reached its lowest concentration at 120
minutes in both normal and hypertensive subjects
(3.71 ± 0.08 and 3.01 ± 0.18 mmol/liter). In normal
subjects, plasma aldosterone was decreased maximally
by 78% at 30 minutes (0.6 ± 0.1 ng/dl) and reached
again the pre-loading values by the end of the test.
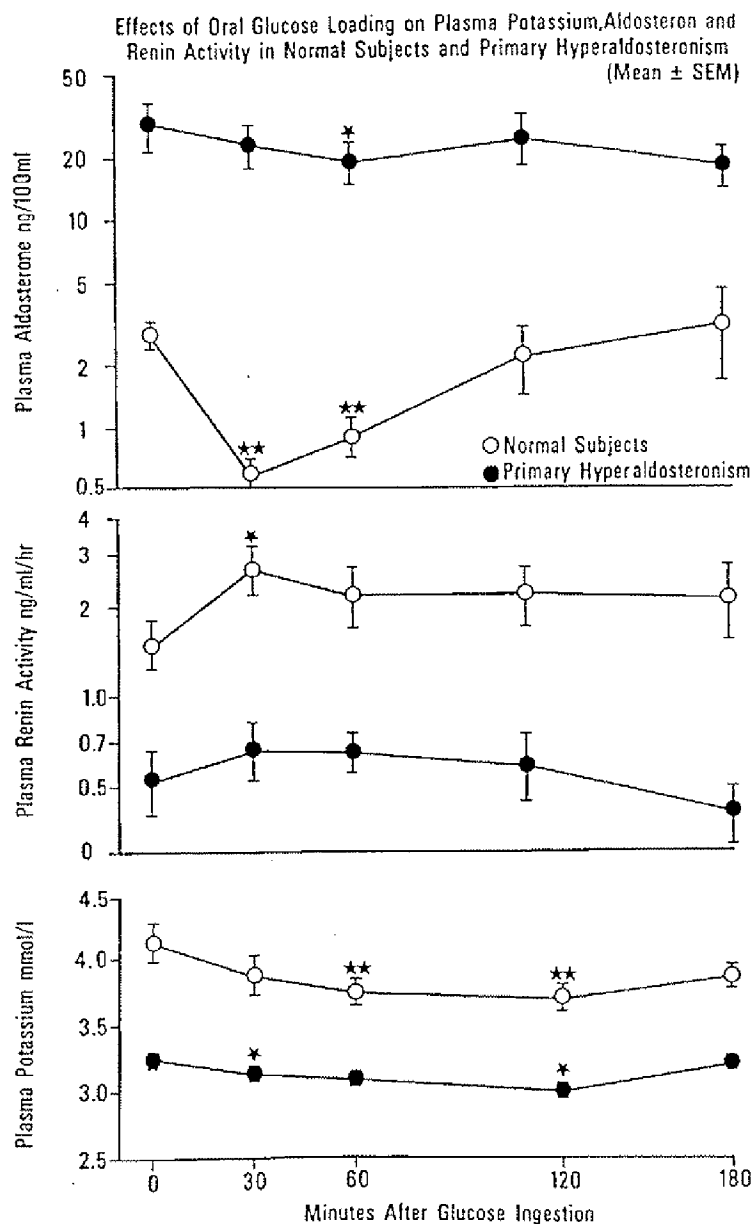
Figure 2:

Symbols and bars indicate Mean ± SEM

* = P<0.05; ** = P<0.01 vs. corresponding basal
value

TABLE 1

Effects of Standard Oral Glucose Loading on Plasma Glucose, Insulin, Cortisol, Potassium, Renin and Aldosterone Levels in Normal Subjects (Group B) (Mean ± SEM).

	Time after glucose loading, min					
	0	15	30	60	120	180
Plasma glucose, mg/dl	91 ± 0.5	116 ± 3.1b	147 ± 5.5c	123 ± 7.6b	103 ± 4.5	86 ± 6.0
Plasma insulin, uE/ml	19 ± 0.9	42.7 ± 3.7b	74.8 ± 7.2c	66.7 ± 6.8c	44.9 ± 3.2b	25 ± 1.7
Plasma cortisol, ug/dl	14.6 ± 0.8	12.8 ± 0.8b	11.7 ± 0.7c	10.7 ± 0.7c	8.7 ± 0.7c	6.9 ± 0.5d
Plasma potassium, mmol/liter	4.42 ± 0.1	4.36 ± 0.09	4.09 ± 0.06c	3.92 ± 0.05c	3.98 ± 0.04c	4.09± 0.05c
Plasma renin activity, ng/ml/hr	1.2 ± 0.1	1.3 ± 0.1	1.9 ± 0.2c	2.0 ± 0.2c	1.8 ± 0.2c	1.5 ± 0.1a
Plasma aldosterone, ng/dl	10.0 ± 1.0	8.0 ± 0.7c	6.4 ± 0.5c	5.8 ± 0.4c	7.2 ± 0.8	9.0 ± 0.9

a = $P < 0.01$; b = $P < 0.005$; c = $P < 0.001$ versus corresponding basal value

In primary hyperaldosteronism, plasma aldosterone was decreased maximally by 30% at 60 minutes (19.5 $\pm$ 4.4 ng/dl), and tended to remain somewhat lowered until the end of the observation period. Plasma renin activity was increased significantly at 30 minutes after glucose loading in normal subjects (2.73 $\pm$ 0.33 ng/ml/hr), while the slight increase noted in primary hyperaldosteronism did not reach statistical significance.

Study B

The effects of glucose loading on plasma glucose, insulin, potassium, renin activity and aldosterone levels in normal subjects of group B were comparable to those observed in group A (Table 1). Furthermore, a progressive decrease (P< 0.001) in plasma cortisol was noted after glucose ingestion.

Study C

In the group with secondary hyperaldosteronism, mean basal and postglucose values of plasma glucose, insulin, potassium and aldosterone did not differ between the normal and borderline hypertensive subjects, therefore allowing combined analysis of these subgroups. Basal plasma glucose, potassium and aldosterone levels in the group with secondary hyperaldosteronism did not differ significantly from the values in the patients with primary hyperaldosteronism; however, plasma insulin was on average significantly (P< 0.001) higher in secondary hyperaldosteronism (Table 2). Sixty minutes following glucose ingestion, plasma potassium and aldosterone levels were significantly (P< 0.01) lower while plasma insulin remained significantly (P< 0.001) higher in secondary than in primary hyperaldosteronism.

TABLE 2

Effects of Standard Oral Glucose Load in Five Normal Subjects and 11 Patients with Secondary hyperaldosteronism* (Mean ± SEM).

	Basal level	Glucose loading	
		60 min	90 min
Plasma glucose, mg/dl	97 ± 1	146 ± 3b	79 ± 1a
Plasma Insulin, µE/ml	27.5 ± 2.1	131.7 ± 4.6b	43.8 ± 2.5
Plasma potassium, mmol/liter	3.20 ± 0.03	2.70 ± 0.03b	3.00 ± 0.04
Plasma aldosterone, ng/dl	26.4 ± 1.1	8.2 ± 0.4b	11.1 ± 0.05a

* induced by four weeks of treatment with the diuretic chlorthalidone (50 mg daily during the first two weeks and 100 mg daily during the second two weeks (11)

$a = P < 0.01$; $b = P < 0.005$ versus corresponding basal value

TABLE 3

Multiple Regression Analysis of Glucose-Induced Changes in
Plasma Aldosterone on Concomitant Changes in Plasma
Glucose, Insulin, Cortisol, Potassium and Renin Levels.

Changes in plasma aldo-sterone versus	Normal Subjects			
	Group A		Group B	
	Dependent variables	R	Dependent variables	R
	PRA	0.89^{c}	PRA	0.53^{a}
	PRA+PK	0.90^{c}	PRA+PI	0.62^{b}
	PRA+PK+PI	0.91^{b}	PRA+PI+PK	0.67^{b}
	PRA+PK+PI+PG	0.91^{b}	PRA+PI+PK+PC	0.73^{c}
			PRA+PI+PK+ PC+PG	0.74^{c}

Primary Hyperaldosteronism

Dependent variables	R
PK	0.69^{a}
PK+PG	0.71^{a}
PK+PG+PRA	0.76^{a}
PK+PG+PRA+PI	0.76^{a}

Multiple correlations were based on mathematical inte-
gration of glucose-induced variations.

PRA = plasma renin activity; PK = plasma potassium;
PG = plasma glucose; PI = plasma insulin; PC = plasma
cortisol.

a = $P < 0.05$; b = $P < 0.005$; c = $P < 0.001$

Multiple Regression Analyses of Studies A and B

Regression analysis was performed using the mathematical integration over time of glucose-induced changes in normal subjects and primary hyperaldosteronism. Changes in plasma aldosterone correlated significantly with the combined changes in plasma renin and potassium. Nevertheless, this regression analysis suggested that the plasma renin activity in normal subjects and plasma potassium in patients with primary hyperaldosteronism explained more of the variability of plasma aldosterone following glucose loading (Table 3).

DISCUSSION

Glucose ingestion induced in both our normal subjects and patients with primary hyperaldosteronism complex endocrine changes involving not only the glucose regulation hormones, but also the renin-angiotensin-aldosterone system. The dynamic pattern observed following glucose-loading was characterized by the expected increase in plasma glucose and insulin concentrations as well as by a significant reduction in the blood levels of potassium and aldosterone. The glucose load also caused a significant increase in plasma renin activity in normal subjects, but not in the patients with primary hyperaldosteronism, whose renin values were suppressed already under basal conditions. These findings suggest that the metabolic changes induced by glucose ingestion are capable of modifying aldosterone secretion in the syndrome of primary hyperaldosteronism.

Plasma potassium and renin are two major factors in the control of aldosterone release (14). Based on multiple regression analysis, changes in plasma aldosterone following glucose-loading were correlated more closely

with variations in renin activity in our normal subjects,
and closer with changes in plasma potassium in primary
hyperaldosteronism. Evidence for a major role of potassium
in mediating glucose-induced decreases in plasma aldoste-
rone has been obtained previously in normal subjects (1,21)
or patients with terminal renal failure (8,32,34). How-
ever, despite the presence of a significant correlation
between glucose-induced changes in plasma aldosterone
and plasma potassium in our previously reported group of
normal subjects (1), the interrelation between these two
factors may not be simply a direct one. The decrease
in plasma aldosterone following glucose loading pre-
ceded a detectable decrement in plasma potassium in
our normal subjects and those of others (12) as well
as in our patients with primary hyperaldosteronism.
On the other hand, a major direct influence of glu-
cose or plasma insulin appears unlikely. Multiple
regression analysis did not reveal any important
contribution of these factors to the variability of
plasma aldosterone in both normal subjects or patients
with primary hyperaldosteronism. This does not exclude
that glucose could play at least a permissive role in
insulin-potassium-mediated aldosterone inhibition (10).
Moreover, additional factors such as corticotropin
could also contribute to glucose-induced aldosterone
suppression. Plasma cortisol levels fell progressively
after glucose load in our normal subjects, but the
contribution of this change to the variability of plas-
ma aldosterone, tested by multiple regression analysis,
was rather small.

In normal subjects, inhibition of the adrenocorti-
cal function by glucose-insulin-induced alterations in
potassium distribution may be counteracted in part by

concomitant renin stimulation (22). The latter response
may be blunted in primary hyperaldosteronism, due to
persistent renin suppression by sodium-fluid volume
retention (6). Moreover, the stimulatory effect of angio-
tensin II on aldosterone secretion tends to be reduced
in such patients (15,35). Therefore, functional impair-
ment of the renin-angiotensin II-mediated pathway for
aldosterone regulation could have contributed to a ten-
dency for prolonged inhibition of plasma aldosterone
following glucose ingestion in our patients as compared
with normal subjects.

Excess aldosterone secretion in primary hyper-
aldosteronism is frequently accompanied by impaired glu-
cose tolerance (7,24). About 50% of such patients have
a pathological response of plasma glucose and insulin
to a standard glucose load (24). In our patients, glu-
cose loading induced higher and more delayed increases
in plasma glucose and smaller increments in circulating
insulin as compared with the normal subjects. The altered
glucose homeostasis has been explained as a consequence
of potassium depletion (7,30), since it can be reversed
by surgical removal of the adrenal adenoma (24). How-
ever, plasma insulin was higher in our subjects with
secondary hyperaldosteronism than in those with primary
hyperaldosteronism, despite a more marked hypokalemia
in the former. This suggests that factors other than
potassium may contribute to a disturbed glucose to-
lerance in primary hyperaldosteronism. Blunted insulin
responsiveness to hyperglycemia could be associated with
a defective control of extracellular potassium (34). In
fact, the decrease in plasma potassium following glucose
load tended to be less pronounced in the patients with
primary hyperaldosteronism (-0.16 $\pm$ 0.09 mmol/liter or

-5 $\pm$ 3%) than in normal subjects (-0.40 $\pm$ 0.08 mmol/liter
or -9 $\pm$ 2%) or in the group with secondary hyperaldo-
steronism (-0.49 $\pm$ 0.12 mmol/liter or -15 $\pm$ 3%). The
magnitude of aldosterone suppression following glucose-
loading was also less marked in primary than in secon-
dary hyperaldosteronism (-30 versus -69%). Considering
the normal responses of plasma insulin and glucose le-
vels in the group with secondary hyperaldosteronism, it
appears possible that limited decreases in plasma
potassium and aldosterone following glucose ingestion
in patients with primary hyperaldosteronism may be re-
lated to their blunted insulin response.

In normal subjects or patients with primary
hyperaldosteronism, plasma levels of aldosterone follow
a circadian rythm (23) and any interpretation should
take the time of sampling into consideration. More-
over, it has been generally recommended that diagnostic
measurements of plasma renin and aldosterone levels
should be performed in the fasting state, mainly be-
cause of a possible mild inhibitory effect of the
fluid (26) and sodium (3) ingested during breakfast.
The demonstration of acute aldosterone suppression and
renin stimulation in response to oral glucose uptake
provides a further rational basis for this policy.
Whether and to what extent such measurements following
glucose loading could gain diagnostic interest, cannot
be answered from the limited number of the present ob-
servations. Nevertheless, the present findings rise the
possibility that measurements of plasma aldosterone
after a standard oral glucose load could help in the
discrimination of primary hyperaldosteronism.

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Insulin response to carbohydrate ingestion after gastric surgery with special reference to hypoglycaemia

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Insulin response to carbohydrate ingestion after gastric surgery with special reference to hypoglycaemia

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SUMMARY   Factors responsible for spontaneous hypoglycaemia were investigated in 23 gastrectomy patients and two patients with vagotomy and pyloroplasty. Plasma insulin and capillary blood glucose levels were followed after giving 175 ml of 50% glucose orally. In gastrectomy patients a significant correlation was found between the height of the peak blood glucose and insulin levels for the same individual. Patients with high peak glucose and insulin levels were significantly more likely to develop hypoglycaemia later in the test. These findings are compatible with the suggestion that the major factor predisposing to hypoglycaemia is a faster than average rate of emptying of the gastric remnant, but this does not explain all the results. In two cases, hypoglycaemia followed an abnormally large insulin response to oral glucose. The results of insulin injection tests in 14 patients do not exclude the further possibility that in some cases hypoglycaemia is due to excessive insulin sensitivity.

Symptomatic hypoglycaemia may occur one and a half to three hours after meals in patients who have undergone partial gastrectomy (Adlersberg and Hammerschlag, 1947; Zollinger and Hoerr, 1947) or vagotomy with a drainage procedure (Tanner, 1966). Only a minority of patients have hypoglycaemic attacks, however, and the reason for this is uncertain. The development of an immunoassay technique (Yalow and Berson, 1960) has made it possible to estimate plasma insulin levels in large numbers of samples. The object of the present study was to use this technique to investigate the difference between those patients with and those without hypoglycaemic symptoms after gastric surgery.

MATERIAL AND METHODS

Twenty-three patients who had undergone partial gastrectomy for benign peptic ulcer were studied (Table). Four of these had also had a truncal vagotomy. In addition, two patients who had had vagotomy with pyloroplasty were investigated, their results being discussed separately from the main group. Ten patients gave a history suggesting spontaneous hypoglycaemia. Twelve gastrectomy patients were studied as outpatients, the remainder being admitted to hospital, usually for investigation of postgastrectomy problems. The patients studied, while not a random sample, represented a spectrum from excellent to bad gastrectomy results. Patients were studied without special dietary preparation. The average daily intake of dietary calories and carbohydrate was estimated for outpatients by a dietician and was measured for those admitted to hospital. Tests were performed in the morning, patients fasting overnight except that outpatients were allowed a sugar-free drink before leaving home. Patients rested sitting for 30 minutes before tests began.

For the oral glucose test, the patient while seated drank 175 ml of 50% glucose rapidly, this being the amount given in earlier studies from this department (Le Quesne, Hobsley, and Hand, 1960). Symptoms were recorded during the test. Blood was drawn at approximately 30-minute intervals for three and a half hours, capillary blood being taken five minutes after venous sampling.

For the insulin sensitivity test, 0·06 unit of soluble insulin per kg body weight was given intravenously and capillary blood taken at 15-minute intervals for one hour.

Blood glucose was measured by the glucose-oxidase method (Huggett and Nixon, 1957). At the height of glucose absorption, the difference between simultaneous capillary and venous levels was found to vary between 10 and 130 mg/100 ml, as previously noted by Mosenthal and Barry (1950). Capillary blood, taken by finger prick after warming the hand, was therefore used for glucose estimations, since this gives a more accurate reflection of the arterial level than venous blood (Jonas, 1933). Plasma insulin was measured on heparinized venous blood by the immunoassay method of Hales and Randle (1963a) using iodinated insulin (^{131}I).[1] Duplicate estimations were

[1] Radiochemical Centre, Amersham.

TABLE

DETAILS OF PATIENTS STUDIED

Patient	Age	Sex	Operation	Normal Diet at Time of Study		Family History of Diabetes	History Suggesting Hypo-glycaemia	Years Since Gastrectomy
				Calories	CHO (g)			
1	59	M	Vagotomy and Polya	1,400	100	—	+	13
2	75	M	Polya	1,600	160	—	—	13
3	43	M	Polya	2,300	300	—	+	8
4	56	F	Vagotomy and Billroth I	1,000	70	—	+	2
5	51	F	Polya	2,200	230	—	+	2
6	55	F	Billroth I	1,300	110	+	—	1
7	52	F	Polya	1,500	200	—	—	10
8	47	M	Polya	1,900	210	—	+	10
9	70	F	Billroth I	2,300	240	—	—	2
10	45	M	Billroth I	2,900	330	—	—	1
11	57	M	Polya	1,000	110	—	—	13
12	45	M	Polya	2,900	380	—	—	2
13	54	F	Polya	1,900	210	—	—	2
14	64	M	Polya	2,700	280	—	—	1
15	39	M	Polya	2,300	230	+	+	12
16	50	M	Billroth I	1,800	180	—	—	2 wk
17	63	M	Vagotomy and Polya	2,100	240	—	+	16
18	47	F	Billroth I	1,600	130	+	—	7
19	55	F	Billroth I	1,600	220	—	+	13
20	59	F	Polya	2,150	175	—	+	3
21	67	F	Polya	640	60	+	—	8
22	47	F	Vagotomy and Polya	3,300	370	+	—	14
23	58	M	Billroth I	1,200	140	—	—	9
24	59	M	Vagotomy and pyloroplasty	1,600	170	—	—	—
25	38	M	Vagotomy and pyloroplasty	?	?	+	+	—

performed on at least two separate assays for every sample.

RESULTS

Most oral glucose tolerance curves showed a high early peak and then a sharp fall, which sometimes continued down to a hypoglycaemic level (Fig. 1). The peak glucose level was reached between 35 and 65 minutes. In three patients the minimum glucose level occurred at 95 minutes, in six at 125 minutes, in seven at 155 minutes, in six at 185 minutes, and in one patient at 215 minutes.

All fasting plasma insulin levels were 20 μU/ml or less. After glucose ingestion, insulin levels usually rose steeply, sometimes following an initial delay. Thereafter, changes in the insulin level closely paralleled changes in the blood glucose. When the blood glucose concentration was lowest the insulin level was at, or rapidly approaching, the fasting level. One gastrectomy patient showed an unusually large insulin response (Fig. 2a). Those with low peak glucose levels had low peak insulin levels and were less likely to have a low blood glucose in the later part of the test (Fig. 2b). Taking the gastrectomy patients as a whole, three significant correlations were found: (a) the higher the peak glucose level, the higher the peak insulin level (Fig. 3); (b) the higher the peak glucose level, the lower the subsequent minimum glucose level (Fig. 4); and (c) the higher the peak insulin level, the lower the minimum

glucose level (Fig. 4). The four patients with both vagotomy and gastrectomy had high peak glucose and insulin levels, but their results did not suggest that vagotomy altered the insulin response to a given glucose level, or altered their sensitivity to endogenous insulin (Figs. 3 and 4). Results in the two patients with vagotomy and pyloroplasty only are shown in Figure 5. One showed a very large, and the other a rather small, insulin response, both being within the range observed in gastrectomy patients.

No correlation was found between the mean daily carbohydrate intake of the 23 gastrectomy patients and either the peak glucose level ($r = 0.0621$), the peak insulin level ($r = 0.0611$), or the minimum glucose level ($r = 0.1636$) on their oral glucose tests.

Many patients had dumping symptoms of varying severity within the first hour after glucose ingestion but hypoglycaemia occurred later after a symptom-free interval. Hypoglycaemic symptoms were reproduced in four of the nine gastrectomy patients and in the one patient with vagotomy and pyloroplasty, who gave a history suggesting spontaneous attacks. One of the 15 patients with no previous history developed hypoglycaemia during the test. The capillary glucose levels of these six patients at the time of hypoglycaemic symptoms were 28, 30, 36, 42, 49, and 60 mg/100 ml. (Venous glucose levels at that time were a mean of 13 mg/100 ml lower.) Observed attacks lasted about 10 to 20 minutes and symptoms included a feeling of undue warmth, sweating, shakiness, dizziness, and difficulty in concentration,
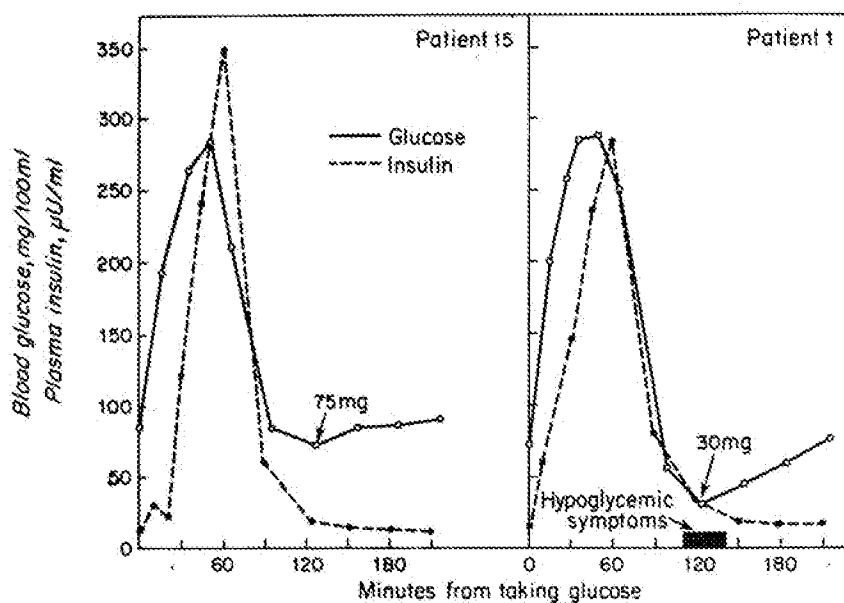
FIG. 1a and b. *Glucose and insulin levels in two gastrectomy patients after ingestion of 175 ml 50% glucose.*
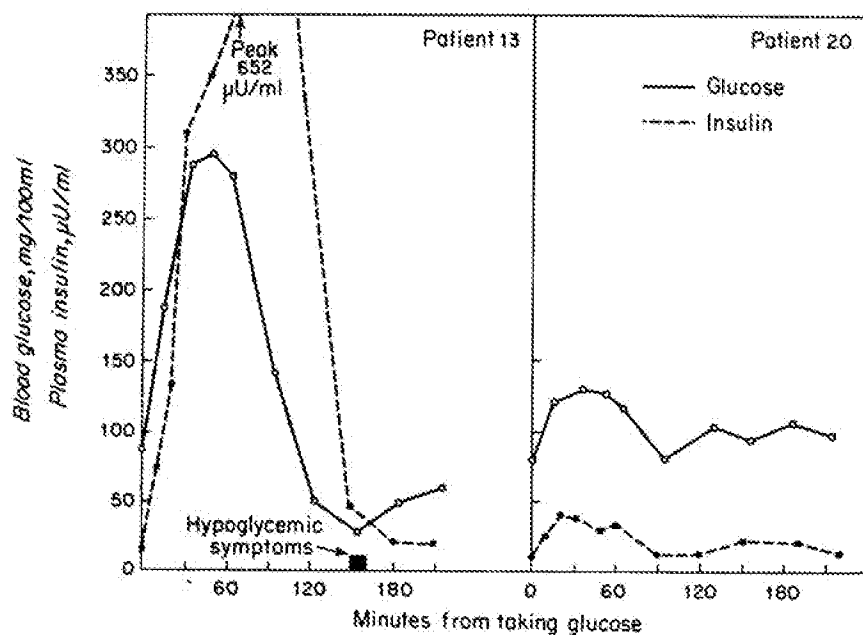
FIG. 2. *Glucose and insulin levels in two gastrectomy patients after ingestion of 175 ml 50% glucose showing (a) an unusually large insulin response and (b) low peak glucose and insulin levels.*
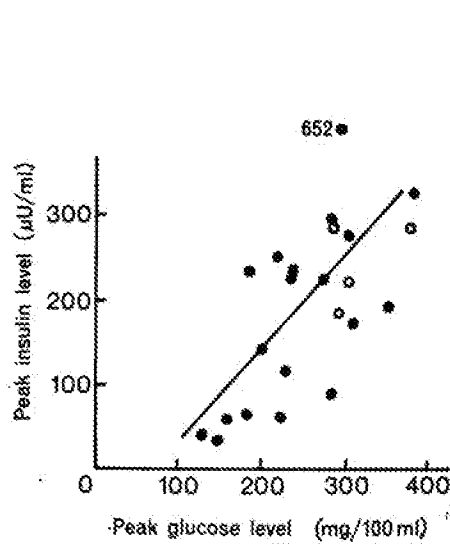
FIG. 3. Correlation between peak glucose and insulin levels in gastrectomy patients after glucose ingestion. $\bigcirc$ = patients after vagotomy and gastrectomy. $r = 0.559$, $p = < 0.005$, $y = 1.07 - 70\,x$.
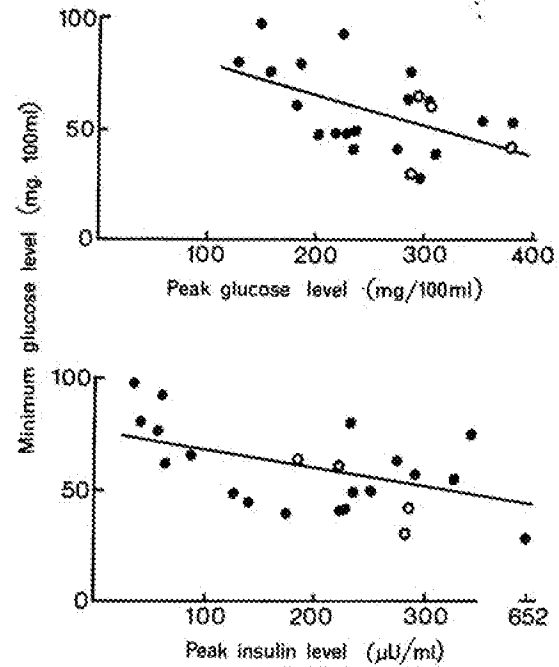
FIG. 4. Above, correlation between early peak glucose and late minimum glucose levels in gastrectomy patients after glucose ingestion. $\bigcirc$ = vagotomy and gastrectomy. $r = 0.5025$, $p = < 0.01$, $y = 93 - 0.133x$.

Below, correlation between early peak insulin and late minimum glucose levels in gastrectomy patients after oral glucose. $\bigcirc$ = vagotomy and gastrectomy. $r = -0.5836$, $p = < 0.005$, $y = 75 - 0.0809x$.

FIG. 5a and b. Glucose and insulin levels in two patients with vagotomy and pyloroplasty showing different insulin responses associated with similar early peak blood glucose levels.

FIG. 6. *Minimum glucose levels after oral glucose and after intravenous insulin in the same 14 gastrectomy patients.* $r = 0.3704$, P $= > 0.1$.
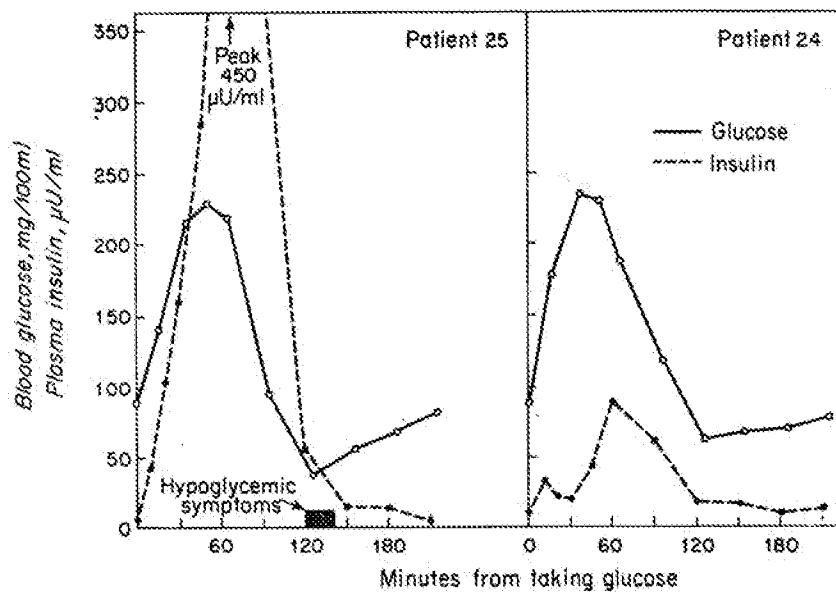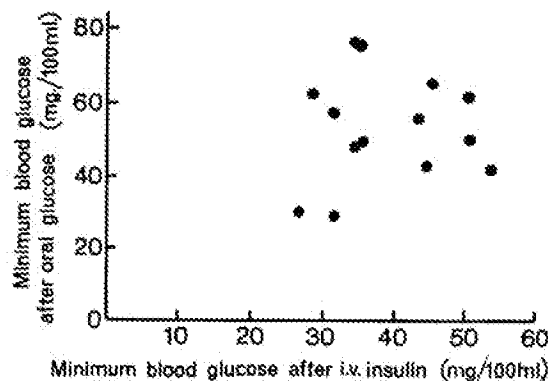
no patient becoming confused or comatose.

Fourteen gastrectomy patients had insulin sensitivity tests (Fig. 6). There was poor correlation between the minimum glucose level after oral glucose and after insulin for the same patient ($r = 0.3704$, P > 0.1) suggesting that differing sensitivity to insulin was not an important factor in determining the occurrence of hypoglycaemia after carbohydrate ingestion.

Gastric remnant emptying was studied in two additional patients given 175 ml of 50% glucose after positioning a nasogastric tube fluoroscopically. At 35 minutes their blood glucose levels were 214 and 263 mg/100 ml and aspiration five minutes later recovered 41 and 13 g glucose respectively.

DISCUSSION

There is some general agreement as to the cause of postgastrectomy hypoglycaemia. The gastric remnant empties faster than the intact stomach (Bruusgaard, 1946) so that ingested carbohydrate is rapidly presented to and absorbed from the small intestine. As a result, peak blood glucose and insulin levels are higher in gastrectomy patients than in normal subjects (Phear, 1962; Roth and Meade, 1965). Excessive insulin release in response to hyperglycaemia is thought to cause subsequent hypoglycaemia (Muir, 1949; Conn and Seltzer, 1955).

Gastrectomy patients given intravenous glucose rarely develop hypoglycaemia (Butler, 1951) probably because the insulin response to intravenous glucose is less than if glucose is absorbed from the intestine (McIntyre, Holdsworth, and Turner, 1964; Elrick, Stimmler, Hlad, and Arai, 1964). However, in the oral glucose tests in the present study, changes in the plasma insulin level closely followed changes

in the blood glucose level suggesting that the latter was the principal factor determining the insulin response.

Variations in the rate and pattern of gastric emptying offer one explanation why only a few gastrectomy patients develop hypoglycaemia. In the oral glucose tests in the present study, patients whose peak glucose and insulin levels were highest were most liable to develop hypoglycaemia later in the test. A rapid initial rate of gastric emptying may cause a higher glucose peak before secreted insulin takes effect. Smith, Fraser, Staynes, and Willcox (1953) found no difference in the emptying rate of a barium-glucose meal between gastrectomy patients with and without hypoglycaemic attacks. However, radiological assessment of gastric emptying is only semi-quantitative, and due to its high specific gravity, barium may pass rapidly through a dependent gastrectomy stoma and so may not accurately reflect the emptying rate of food or a glucose test meal. Gastric emptying tests on two of our patients confirmed that the gastric remnant has considerable reservoir function and suggested that the emptying rate of hypertonic glucose can vary considerably. Also, although we have no direct evidence for this, the tail end of a meal is perhaps sometimes still leaving the gastric remnant at two to three hours, thus protecting against hypoglycaemia. This could explain the difference in minimum glucose levels between the patients whose tests are illustrated in Figure 1.

An unusually large insulin response to oral glucose was seen in two patients developing hypoglycaemia during the test, one with a gastrectomy (no. 13) and one with a vagotomy and pyloroplasty (no. 25). The first patient had never had such symptoms before, whereas the other had frequent, troublesome attacks. This suggests that hypoglycaemia is sometimes due to an excessive insulin response to a given blood glucose level, but that this may not be a consistent occurrence in the same individual.

In our patients with the combined operation of vagotomy and partial gastrectomy the insulin response to a given blood glucose level and the hypoglycaemic effect of endogenous insulin were unremarkable. The higher incidence of hypoglycaemic symptoms reported after vagotomy with a drainage procedure as compared with partial gastrectomy (Goligher, Pulvertaft, De Dombal, Conyers, Duthie, Feather, Latchmore, Shoesmith, Smiddy, and Willson-Pepper, 1968) are perhaps related to faster gastric emptying after vagotomy (McGill, Cameron, Hobsley, and Le Quesne, 1968) rather than to any direct effect of vagotomy on insulin release or action.

Increased sensitivity to exogenous insulin could not be demonstrated statistically in our gastrectomy

patients developing hypoglycaemia after oral glucose. However, the two patients with the lowest minimum glucose levels after oral glucose were more sensitive than most to injected insulin. Barnes (1947) suggested that postgastrectomy hypoglycaemia might be due to increased insulin sensitivity, but Muir (1949) found increased sensitivity to injected insulin in only one of 12 patients with hypoglycaemia after oral glucose. Smith *et al* (1953) found a delay in recovery from hypoglycaemia after insulin injection in patients with a history of hypoglycaemic attacks, although the mean minimum glucose level reached was not as low as in gastrectomy patients without attacks. We are inclined to agree with Muir (1949) that increased insulin sensitivity cannot be eliminated as a factor predisposing to hypoglycaemia in some cases.

On a low carbohydrate diet, normal subjects show impaired oral glucose tolerance, reduced sensitivity to injected insulin (Himsworth, 1935), and a smaller plasma insulin response to oral glucose (Hales and Randle, 1963b). Many of our patients could not tolerate a full carbohydrate diet because of dumping symptoms and tests were therefore performed without special dietary preparation. No correlation was found between the dietary carbohydrate content and either the peak insulin level or the peak or minimum glucose levels after oral glucose. The previous diet was not, therefore, a significant factor in determining the shape of the glucose tolerance curves in these gastrectomy patients. It was noted that patients with a history of hypoglycaemia included both those who had restricted food intake due to fear of dumping attacks, and those who were able to eat heartily.

Hypoglycaemia occurring several hours postprandially may be due to pre-diabetes (Seltzer, Fajans, and Conn, 1956). However, a family history of diabetes, which would increase the chance of the patient having pre-diabetes, was found about as frequently in those with as those without hypoglycaemia in the present series.

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1975 ANNUAL REPORT AVAILABLE

The Association's Annual Report for 1975, reflecting the ADA's growth in professional, patient, and public information and research allocations, which reached a new high of $1,300,000 for the year, is available free from the national office. This sum is four times the total of two years previously.

NEWS OF AFFILIATE ASSOCIATIONS

The University of Michigan, in cosponsorship with the ADA Michigan Affiliate, will hold a Conference on Diabetes Mellitus on October 12 and 13, 1976. Current concepts on the etiology, pathogenesis, and management of the disease and its complications will be explored. For further information write Robert K. Richards, Ph.D., Office of Continuing Education, G-1109 Towsley Center, University of Michigan Medical Center, Ann Arbor, Mich. 48109.

NEWS NOTES

EUROPEAN ASSOCIATION MEETING

The Twelfth Annual Meeting of the European Association for the Study of Diabetes will be held in Helsinki, Finland, September 1-3, this year. Inquiries should be made to the 12th EASD Meeting Secretariat, Box 2, SF—00290, Helsinki.

INTERNATIONAL CONGRESS

All sessions of the International Diabetes Federation's Ninth Congress are scheduled for the Science Hall building, Vigyan Bhavan, New Delhi, India, October 31-November 5. Travel arrangements may be secured through Garber Travel, 1406 Beacon St., Brookline, Mass., 02146. Phone 617-734-2100.

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Plasma Glucose and Insulin Responses to Orally Administered Simple and Complex Carbohydrates

Phyllis A. Crapo, R.D.,* Gerald Reaven, M.D.,†
and Jerrold Olefsky, M.D.,‡ Palo Alto

SUMMARY

We have studied the effects of glucose, sucrose, and various starches on postprandial plasma glucose and insulin responses in 19 subjects. All carbohydrate loads were calculated to contain 50 gm. of glucose, and the response to each carbohydrate was tested twice: when given alone in a drink or when given in combination with other nutrients as a meal. The data demonstrate: (1) Glucose and sucrose elicited similar plasma glucose response curves, but sucrose elicited a somewhat greater (20 per cent) plasma insulin response. (2) Raw starch ingestion resulted in a 44 per cent lower glucose response and a 35-65 per cent lower insulin response than did either glucose or sucrose ingestion. (3) When carbohydrate was given as a meal the plasma glucose responses were 40-60 per cent lower than when the same carbohydrate was given as a drink, while the insulin responses were generally similar, and (4) when different cooked starches were compared, the plasma glucose and insulin responses to rice were significantly lower (50 per cent) than to potato. In conclusion, the size of the carbohydrate molecule appears to influence the postprandial glucose and insulin responses such that more complex carbohydrates (starches) elicit lower responses. This effect may be related to differences in digestion rather than to differences in absorption. DIABETES 25:741-47, September, 1976.

The carbohydrate content of diets has received considerable attention in recent years. Diets restricted in carbohydrate have been recommended for two particular groups of patients—those with diabetes[1] and those with endogenous hypertriglyceridemia.[2] The rationale for carbohydrate restriction is usually based on the supposition that a decrease in the carbohydrate content of the diet will decrease the postprandial glucose and/or insulin responses. The usual way of accomplishing total carbohydrate reduction is to decrease the amount of glucose and refined sugar (sucrose) without necessarily decreasing the starch (complex carbohydrate) content of the diet. This is based on the belief that glucose and sucrose are more readily available for immediate absorption, thereby producing a greater and faster rise in postprandial plasma glucose and insulin responses, in contrast to the supposedly slower and more gradual digestion and absorption of more complex carbohydrates.[3-5]

However, other lines of investigation suggest that starch and glucose are assimilated at the same rate. Thus, after a test meal in normal man, Dahlqvist and Borgstrom[6] found that sufficient amounts of intraluminal amylase were present to rapidly hydrolyze ingested starch. This has been confirmed by Fogel and Grey,[7] who found that absorption, not intraluminal digestion, was the rate-limiting step in over-all starch assimilation. These findings cast doubt on the original

assumption that the kind of dietary carbohydrate affects the subsequent postprandial serum glucose response. In addition, starch tolerance tests have been used to diagnose amylase deficiency in patients with pancreatic insufficiency,[8-10] and these tests show that in normals the increases in blood glucose following starch or glucose are comparable. However, these studies[8-10] were done with the carbohydrate as a single agent mixed with water, and it is conceivable that some interplay between food constituents (protein, fat, carbohydrate) occurs that could result in different rates of digestion and absorption for different carbohydrates. Furthermore, since the postprandial plasma insulin response is partially dependent on the ability of food constituents to stimulate the secretion of a number of gastrointestinal insulinogenic hormones,[11] it is possible that when mixed with other nutrients (protein and fat) different kinds of carbohydrate differ in their ability to stimulate these gastrointestinal insulinogenic factors.

We have therefore studied the effects of different kinds of dietary carbohydrate on the postprandial plasma glucose and insulin responses when given alone and in combination with other nutrients in a test meal.

MATERIALS AND METHODS

Nineteen normal volunteers, eleven women and eight men, were studied. Mean age of the 19 subjects was 34 (range: 25-48) years. Mean weight was 147 pounds, and mean relative weight according to the Metropolitan Life Tables was 0.92, with a range of 0.78 to 1.15. No drugs known to affect glucose or insulin metabolism were being used by any of the

subjects. During the course of the studies, each subject consumed a weight-maintenance solid-food diet that included at least 200 gm. of carbohydrate each day. Three different sources of carbohydrate (glucose, sucrose, and starch) were studied. Each was studied alone and in combination with other nutrients. Since starch is normally eaten in its cooked form, and our starch solution was uncooked, we also studied potato and rice. The composition of these test carbohydrate loads is outlined in table 1. It should be noted that we used 100 gm. of sucrose, 50 gm. of glucose, and 50 gm. of starch. The test loads were calculated so that the glucose load in all three carbohydrates would be equal. Throughout the rest of the paper the carbohydrates when given alone will be referred to as "drinks" and when given with the other nutrients will be referred to as "meals." All tests were conducted following an overnight fast, and their order was randomized. At 8 a.m., the subject was given one of the solutions or the potato or rice to drink or eat. They consumed the test load in 15 minutes. Blood samples were drawn for measurement of plasma glucose and insulin at time 0 and at 30, 45, 60, 120, and 180 minutes following the beginning of the period of consumption. There was at least a one-day interval between each study.

ANALYTIC METHODS

Samples for plasma glucose were collected in EDTA tubes and measured by a Beckman Glucose Analyzer by the glucose oxidase method of McComb and Yushok.[12] Plasma immunoreactive insulin was measured by the method of Desbuquois and Aurbach.[13] Statistical analysis was carried out by the use of the paired t-test for dependent means.

TABLE 1

Composition of tolerance tests

	Glucose gm.	Sucrose gm.	Soluble starch* gm.	Powdered egg albumin gm.	Corn oil gm.	Russet potato gm. (raw)	Long-grain white rice gm.	Lemon extract	Total volume
A. Glucose (drinks)	50	—	—	—	—	—	—	5 ml.	500 ml.
B. Sucrose (drinks)	—	100†	—	—	—	—	—	5 ml.	500 ml.
C. Starch (drinks)	—	—	50	—	—	—	—	5 ml.	500 ml.
D. Glucose (meals)	50	—	—	20	20	—	—	5 ml.	500 ml.
E. Sucrose (meals)	—	100	—	20	20	—	—	5 ml.	500 ml.
F. Starch (meals)	—	—	50	20	20	—	—	5 ml.	500 ml.
G. Potato (baked)	—	—	—	—	—	385 ‡	—	—	500 ml.//
H. Rice (boiled)	—	—	—	—	—	—	61.5§	—	500 ml.//

*Amylopectin, Sigma.
†100 gm. of sucrose was used to give equivalent 50 gm. load of glucose.
‡385 gm. of raw Russet potato was baked and the skins were removed. Mean carbohydrate ingested was 51 gm.
§61.5 gm. of dry rice contains 50 gm. of carbohydrate.
//Subjects drank 500 cc. water while eating the potato or rice.

RESULTS

The plasma glucose and insulin responses to the glucose, sucrose, and starch drinks are summarized in figure 1. It can be seen (figure 1A) that the plasma glucose curve following a 50-gm. glucose load is not statistically different from the plasma glucose curve following a 100-gm. sucrose load (50 gm. glucose). The plasma glucose response curve to starch is flat, but because the glucose and sucrose curves decrease below fasting values at later time points, it exceeds the curves for glucose and sucrose at 60, 120, and 180 minutes. The plasma insulin curve (figure 1B) following the sucrose load is greater at all points than the plasma insulin curve after glucose, and these differences are statistically significant at 30 (p < 0.01) and 120 (p < 0.01) minutes, indicating that sucrose results in a greater insulin response than does glucose on a molar basis. The insulin response curve following the starch is flat, but it exceeds the glucose and sucrose curves at 120 and 180 minutes.

Figure 2 compares the plasma glucose and insulin

FIG. 2. Mean (± S.E.) plasma glucose (A) and insulin (B) responses to glucose, sucrose, and starch meals.

responses to the glucose, sucrose, and starch meals. There is no difference in the glucose response to a glucose or sucrose meal (figure 2A). The glucose response to a starch meal is flattened in the first hour compared with glucose and sucrose meals but, again, is higher at 60, 120, and 180 minutes. The insulin response (figure 2B) following the sucrose meal is statistically greater than following the glucose meal at 120 (p < 0.005) and 180 (p < 0.005) minutes, and again it can be seen that, on a molar basis, sucrose results in a greater insulin response than does glucose. Both the glucose and sucrose meals have significantly greater insulin responses than do starch meals.

In figures 3 and 4 one can see that although identical amounts of carbohydrate were given in the drinks and meals, the plasma glucose response (figure 3) to meals was always less than that to drinks, while the insulin responses (figure 4) were slightly higher with the meals. We have reported similar findings previously,[14] and this indicates that the interplay of food constituents will attenuate glucose responses without affecting over-all insulin responses.

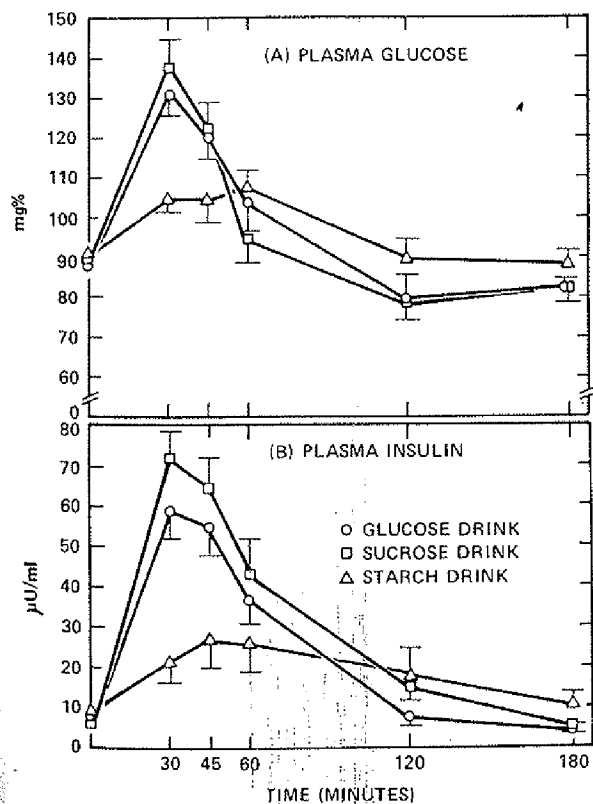The plasma glucose and insulin response curves to the starch drinks and starch meals are quite flat, and

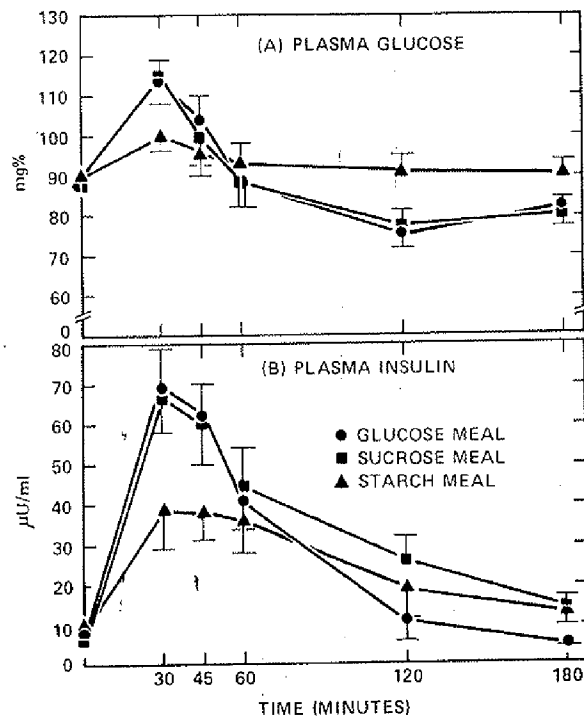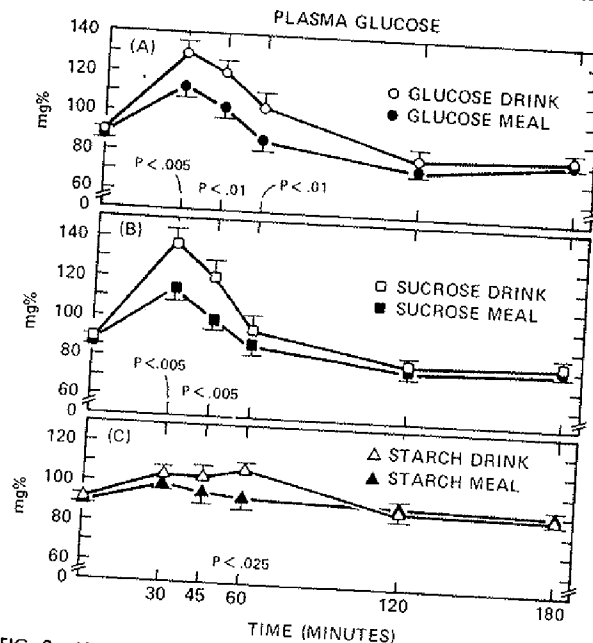FIG. 1. Mean (± S.E.) plasma glucose (A) and insulin (B) responses to glucose, sucrose, and starch drinks.

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FIG. 3. Mean (± S.E.) plasma glucose responses to glucose (A), sucrose (B), and starch (C) given as meals or drinks.

we measured free glucose in our potato samples found it to be negligible.

To facilitate cross-comparisons of the glucose insulin responses to the various stimuli, the respo to each of the oral carbohydrate loads are summar in table 2.

DISCUSSION

We have studied the effects of orally administe simple and complex carbohydrates on the postpran glucose and insulin responses when given alone i drink and when given in combination with other n rients in a test meal. The results show that the plas glucose and insulin responses following oral gluco either drinks or meals, are higher than the respon: following starch ingestion. These results seem to su port earlier studies showing that simple carbohydrat cause a greater and faster rise in postprandial gluco and insulin responses than did the more complex ca bohydrates. Thus, Allen[3] found that starches cause less glycosuria in diabetic dogs than did glucos while Conn and Newburgh[4] demonstrated a high blood glucose response to oral glucose than to an equiv alent load of starch given as potato or bread in diabet ic subjects. Furthermore, Swann et al.[5] demonstrate that the blood glucose and insulin responses wer

we felt that this could possibly be related to using an uncooked starch. Although amylases can attack raw starch, the gelatinization of starch during cooking may make the starch more accessible to the enzymes and the action much more rapid. Therefore, we decided to try some sources of starch that were cooked and were more physiologic in humans. Plasma glucose and insulin responses were determined when starch was given as cooked potato or rice. As demonstrated in figure 5, the glucose and insulin responses to potato were significantly greater than the glucose and insulin responses to rice,§ although on comparing figures 1 and 5 (also table 2), one can see that the glucose and insulin responses to potato are still less than the responses to the glucose drink. These results were surprising and indicate a difference in the ability of different starches to elicit glucose and insulin responses. Since potatoes are active metabolically and convert starch to sugar during storage, it seemed possible that small amounts of free glucose ingested with the potato starch could affect the postprandial glucose and insulin responses. However, this was not the case, since

§In this case, 13 patients had rice tests and 17 patients had potato tests, and the data in figure 5 represent the mean (± S.E.) of all tests. However, for the paired statistical analysis only the results from the 13 patients who had both tests were used.
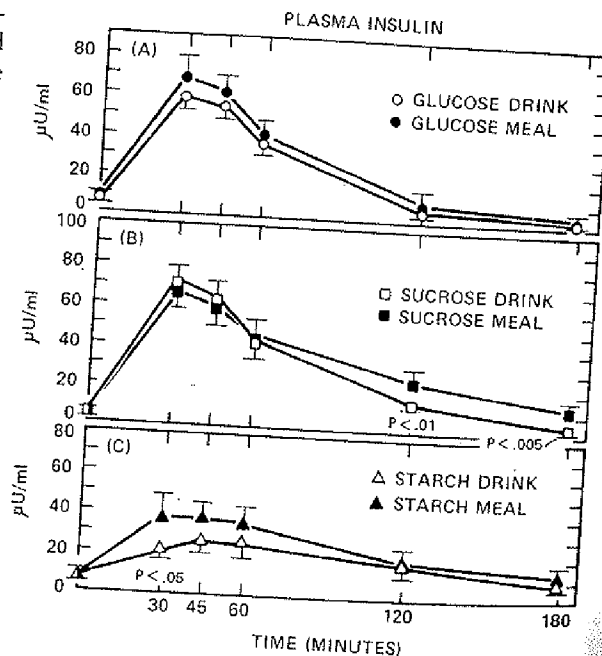

FIG. 4. Mean (± S.E.) plasma insulin responses to glucose (A), sucrose (B), and starch (C) given as meals or drinks.

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(A) PLASMA GLUCOSE

mg %

P < .025
P < .005   NS   P < .01   NS

(B) PLASMA INSULIN

μU/ml

● POTATO
○ RICE

P < .025
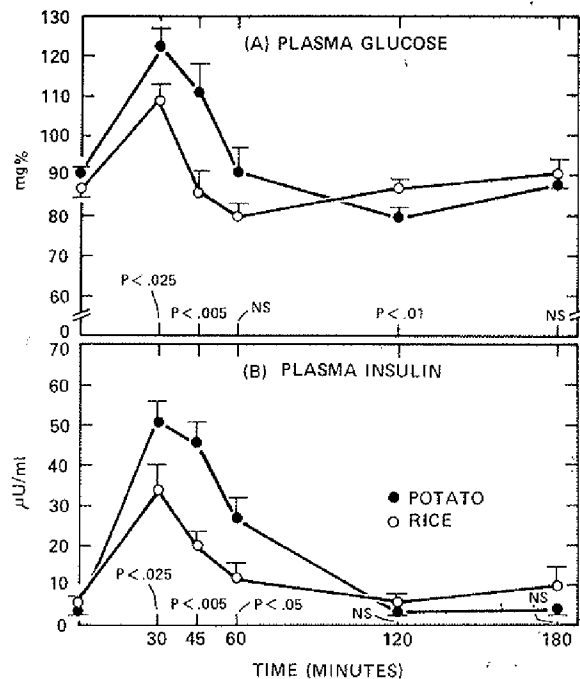P < .005   P < .05   NS   NS

30 45 60   120   180
TIME (MINUTES)

FIG. 5. Mean (± S.E.) plasma glucose (A) and insulin (B) responses to 50 gm. of glucose given as potato or rice.

higher following a glucose load than following a starch load.

The above results seem to be somewhat paradoxic in view of recent direct studies of digestive and absorptive processes. For example, Dahlqvist and Borgstrom[6] and Fogel and Grey[7] have demonstrated that intraluminal amylase is present in excess and that ingested starch is rapidly hydrolyzed following a test meal. These workers have concluded that enzymatic digestion is not the rate-determining step and that glucose and starch are absorbed as glucose at the same rate. Our results are not necessarily inconsistent with these latter findings, since some other effect, such as the rate at which starch or its digestive products enter the small intestine, may also be responsible for the lower glycemic and insulin responses following starch ingestion. In addition, it is possible that the uncooked state of the starch we employed in the starch drinks and meals was a factor in the results, since Althausen and Uyeyama[8] have reported that "soluble starch" resulted in flat glucose curves if mixed with cold water but normal glucose curves if mixed with boiling water. Humans normally eat their starch or complex carbohydrates in a cooked state, and it is possible that the cooking of the carbohydrate makes a critical difference in its fate in the gastrointestinal tract.

The idea that digestion and absorption may not be the same for all starch molecules receives support from the data presented in figure 5. Rice starch elicited a much lower insulin and glucose response than did potato starch, and this has obvious potential importance in formulating rational dietary-therapeutic programs for patients in whom carbohydrate restriction is advised. The mechanism for these differences is not clear, although gastric emptying time and the physical availability of the starch to the hydrolytic enzymes may be factors.

Since our results indicate that ingestion of a large carbohydrate molecule results in lower plasma glucose

TABLE 2

Glucose and insulin responses during the oral carbohydrate tolerance tests

	F	30′	45′	60′	120′	180′
			Plasma glucose (mg. per cent)			
1. Glucose drink (N=19)	90 ± 1.1*	131 ± 5.1	121 ± 6.3	104 ± 6.9	80 ± 5.7	83 ± 4.1
2. Sucrose drink (N=19)	89 ± 1.3	138 ± 7.3	121 ± 8.1	95 ± 6.5	79 ± 4.4	82 ± 2.7
3. Starch drink (N=7)	91 ± 1.7	105 ± 2.8	104 ± 5.3	108 ± 4.3	90 ± 5.4	89 ± 2.7
4. Glucose meal (N=19)	89 ± 1.1	114 ± 5.5	104 ± 6.0	88 ± 6.0	75 ± 3.1	82 ± 1.8
5. Sucrose meal (N=19)	89 ± 1.1	115 ± 7.0	100 ± 6.2	88 ± 5.9	77 ± 3.5	80 ± 2.5
6. Starch meal (N=7)	89 ± 0.9	100 ± 3.6	96 ± 5.6	93 ± 5.2	91 ± 3.7	90 ± 3.1
7. Rice (N=13)	87 ± 2.0	109 ± 4.1	86 ± 4.6	80 ± 3.1	87 ± 2.4	91 ± 2.7
8. Potato (N=17)	91 ± 1.2	123 ± 4.1	111 ± 7.2	91 ± 6.0	80 ± 1.8	88 ± 1.2
			Plasma insulin (μU./ml.)			
1. Glucose drink (N=19)	6 ± 1.2	59 ± 6.8	55 ± 6.9	37 ± 6.0	8 ± 1.7	4 ± 0.8
2. Sucrose drink (N=19)	6 ± 1.2	72 ± 6.7	64 ± 8.3	43 ± 8.6	15 ± 3.1	5 ± 1.1
3. Starch drink (N=7)	8 ± 1.3	21 ± 4.7	27 ± 6.5	26 ± 7.3	18 ± 7.0	10 ± 3.5
4. Glucose meal (N=19)	7 ± 1.4	69 ± 10.6	62 ± 7.8	41 ± 7.3	11 ± 4.5	5 ± 1.2
5. Sucrose meal (N=19)	6 ± 1.1	67 ± 8.5	60 ± 9.8	45 ± 8.7	26 ± 5.7	14 ± 3.3
6. Starch meal (N=7)	9 ± 2.5	39 ± 9.9	38 ± 7.1	36 ± 7.8	19 ± 6.8	14 ± 4.3
7. Rice (N=13)	6 ± 1.6	34 ± 5.8	20 ± 4.0	12 ± 3.9	6 ± 1.6	10 ± 5.1
8. Potato (N=17)	4 ± 0.7	51 ± 4.7	46 ± 5.1	27 ± 5.5	4 ± 0.9	4 ± 0.9

*Mean ± Standard Error.

and insulin responses, one might expect sucrose ingestion to result in lower responses than glucose ingestion, since sucrose is a disaccharide and requires enzymatic digestion prior to absorption. However, it has been shown that disaccharide digestive processes provide monosaccharides in sufficient quantities for transport to occur at maximal rates, and in both man[15] and rats[16] the rate of absorption of glucose and fructose components did not differ whether sugar was fed as sucrose or as its monosaccharide mixture. Our results are compatible with these latter findings, since we found that when given in equimolar amounts, glucose and sucrose result in equal glycemic responses. However, sucrose did elicit a greater insulin response than glucose; the reason for this is not clear, since sucrose consists of glucose and fructose, and fructose alone does not stimulate insulin secretion.[17] On the other hand, Curry et al.[18] have shown that fructose can potentiate insulin release in the presence of glucose; this could possibly account for the increased insulin response to sucrose seen in figures 1 and 2. In addition, it is possible that some conversion of fructose to glucose may occur within the intestinal mucosa, resulting in a larger absolute load of glucose in the plasma with the sucrose than with the glucose.[19,20] This increased load could account for the increased insulin secretion.[21]

Our findings that starches generally give lower glucose responses than pure glucose while the disaccharide (sucrose) gives an equivalent response might be explained by examining the digestive processes. Starch digestion proceeds by a two-step process: the starch molecule is hydrolyzed (amylase) to disaccharides (maltose) and dextrins in the lumen of the gut; these products are then converted to monosaccharides by disaccharidases located on the brush border of mucosal cells. Thus, our results suggest that the brush-border phase of digestion does not limit glucose absorption whereas the luminal phase might. Clearly, slowed luminal digestion could result from delayed gastric transit time, differences in fiber content, or anything that causes the starch molecule to be physically unavailable to the luminal enzymes and need not imply deficient enzyme activity per se.

Lastly, when glucose, sucrose, and starch were given as meals, lower glucose but similar insulin responses resulted than after plain drinks. We have reported similar findings previously,[14] and Estrich et al.[22] have also shown that in adult diabetics the addition of protein and fat to a carbohydrate load leads to flattening of the glucose response curve. It is possible

that these differences are due to stimulation of gastrointestinal insulinogenic hormones by protein[23] or that the amino acids derived from protein have potentiated beta-cell secretion of insulin.[24] Additionally, it has been well demonstrated that fat has an inhibitory effect on gastric emptying.[25] Thus, since the glycemic response to a meal depends on the rate of entry and removal of glucose from plasma, the lower glycemic response to meals could be due to slower absorption, as a result of delayed gastric emptying, or enhanced removal due to insulin's stimulatory effect on glucose uptake.

In conclusion, we find that complex carbohydrates (starches) result in lower glucose and insulin responses than equivalent amounts of glucose as mono- or disaccharides. The mechanisms for this effect are not clear, although delayed luminal digestion seems likely. Finally, the unexpected finding that rice starch resulted in lower plasma glucose and insulin responses than potato starch indicates that all starches are not treated identically by gastrointestinal digestive and absorptive processes. Clearly, further studies will be necessary to delineate the mechanism for this latter finding.

ACKNOWLEDGMENTS

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EFFECT OF 50 AND 100 g GLUCOSE LOADS ON PERIPHERAL MUSCLE GLUCOSE METABOLISM IN NORMAL MAN

Effets sur le métabolisme glucosé du tissu musculaire périphérique de l'homme sain d'une charge orale de 50 ou 100 g de glucose

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RÉSUMÉ

L'étude a pour but de comparer les effets d'une charge orale de 50 g (G.50) ou de 100 g (G.100) de glucose sur la consommation de glucose par les muscles de l'avant-bras ainsi que sur les composantes du métabolisme oxydatif et non-oxydatif chez l'homme normal. Dix sujets sains, de sexe masculin, ont été étudiés le matin à jeun (après 12-14 h de jeûne) et pendant les 3 heures suivant l'ingestion du glucose. Le métabolisme périphérique du glucose a été étudié en utilisant la technique de l'avant-bras pour mesurer les échanges musculaires du substrat couplée à la calorimétrie indirecte.

La quantité de glucose captée par les muscles, de même que celle métabolisée par la voie non-oxydative, sont plus importantes dans le test G.100 que dans le test G.50 (G.100 : 178,9 ± 19,7 et 155,3 ± 23,0 vs 103,5 ± 16,6 et 85,2 ± 16,7 mg/100 g muscles de l'avant-bras par 3 heures, respectivement). L'oxydation du glucose par les muscles n'est pas significativement différente après les 2 charges de glucose. Les concentrations plasmatiques d'insuline sont significativement plus élevées après 100 g de glucose qu'après 50 g de glucose.

En conclusion, cette étude révèle une réponse métabolique dose-dépendante en ce qui concerne l'utilisation musculaire et le métabolisme non-oxydatif du glucose après l'ingestion d'une charge de 50 g et de 100 g de glucose chez le sujet normal. Par contre, la réponse de la voie oxydative ne semble pas être directement proportionnelle à l'importance de la charge orale de glucose.

Diabete Metab., 1992, *18*, 78-83.

Mots clés : Métabolisme périphérique du glucose. Captation du glucose par l'avant-bras. Métabolisme oxydatif et non-oxydatif du glucose-charge orale de 50 et 100 g de glucose.

SUMMARY

The present study was designed to determine the effects of 50 and 100 g glucose loads on forearm muscle glucose uptake, oxidation and nonoxidative glucose metabolism in normal man. Ten healthy male subjects were studied during the postabsorptive state (12-14 h overnight fast) and for 3 hours following glucose ingestion. Peripheral glucose metabolism was analysed by the use of the forearm technique to estimate muscle exchange of substrate combined withy indirect calorimetry.

Greater forearm muscle uptake and nonoxidative metabolism of glucose were observed in the subjects during the G.100 study than during the G.50 study (G.100 = 178.9 ± 19.7 and 155.3 ± 23.0 vs 103.5 ± 16.6 and 85.2 ± 16.7 mg/100 g forearm muscle. 3 h, respectively). the muscle glucose oxidation dit not show significant difference after the two glucose loads. Insulin levels reached after 100 g glucose ingestion were significantly higher than after the 50 g glucose load.

In conclusion, this study revealed a dose-dependent metabolic response in the muscular tissue of normal subjects to oral glucose loads of 50 and 100 g, with respect to forearm muscle glucose uptake and nonoxidative glucose metabolism. The oxidative responses of the muscle tissue to the oral glucose challenges seem not to be directly proportional to the ingested meals.

Diabete Metab., 1992, *18*, 78-83.

Key words : Peripheral glucose metabolism. Forearm glucose uptake. Oxidative and nonoxidative glucose metabolism. 50 and 100 g oral glucose disposal.

The quantitative participation of the insulin-dependent tissues in the disposal of an oral glucose load has been intensively investigated, emphasizing the important role of peripheral tissues (particularly the muscle) in the processing of this meal (1-4). In a previous study (5), we showed that in normal subjects after ingestion of 75 g of glucose, 30.3 ± 2.1 g of this substrate were taken up by the skeletal muscle in the whole body and 8.1 ± 0.6 g were oxidized in this tissue during 3 hours. However, the magnitude of this metabolic response in relation to the size of the glucose load was not defined in these studies. The characterization of this dose-response relationship is important because different glucose loads are used in oral tests of glucose tolerance, which are widely employed for the diagnosis of diabetes mellitus and other forms of glucose intolerance.

The present study was thus designed to determine forearm muscle glucose uptake, oxidation and nonoxidative glucose metabolism in the postabsorptive state and during the 3 hours following the ingestion of 50 g or 100 g glucose loads in normal control subjects. Peripheral glucose metabolism was analysed by using of the forearm technique to estimate muscle exchange of substrate combined with indirect calorimetry.

SUBJECTS AND METHODS

Ten healthy male subjects between 23 and 39 years of age (mean age, 28.5 ± 1.9 years) were studied at the University Hospital of the School of Medicine of Ribeirão Preto (Brazil). Their mean body weight was 66 ± 4 kg, or 99 ± 3 % of ideal body weight as determined by the Metropolitan Life Insurance Tables (1959). There was no evidence of liver disease or diabetes mellitus, as indicated by normal laboratory tests and oral glucose tolerance tests (6). All subjects were asked to take meals containing a minimum of 200 g carbohydrate for at least 3 days prior to the study. At this time, all participants were free from acute illnesses and any medication. Before giving their free consent to participate, the volunteers were carefully informed of the nature, purpose, and possible risks of the study. No side effects were observed in any of the studies. The protocol was approved by the Ethical Committee of the University Hospital — School of Medicine of Ribeirão Preto — USP.

After a overnight fast 12-14 h, the studies were initiated in the morning with the subjects resting in a bed in the supine position. The brachial artery in the left arm and a right antecubital deep vein were cannulated as previously described (5, 7, 8). After an equilibration period of 30-60 minutes, forearm blood flow was determined by capacitance plethysmography (8, 9), and arterial and venous blood samples were drawn simultaneously to determine plasma glucose (10), FFA (11), total blood CO_2 and O_2 (12) and serum insulin (13). After this procedure under basal conditions, the subjects ingested 50 g or 100 g of glucose dissolved in 300 ml of flavoured water, and further blood samples were collected and forearm blood

flow determinations made at 30, 60, 120, and 180 minutes after glucose ingestion. A second study with the alternate glucose load was performed no more than 4 weeks later under the same experimental clinical conditions.

The amount of substrate taken up or released by the forearm in a determined time (Q) was calculated using the following equation: $Q = F (A-V)$, where F is the forearm blood flow, and A and V are the arterial and venous concentrations of the substrate, respectively. Forearm glucose uptake rate was calculated using blood flow and whole blood arterial and venous glucose concentrations. Whole blood glucose concentration was calculated from the respective plasma glucose level by the formula: whole blood glucose concentration = plasma glucose concentration x (1 − 0.3 hematocrit) (14). The relationship between the arteriovenous differences in CO_2 and O_2 was used to determine the respiratory quotient (RQ). The Lusk tables (15) were then used to obtain the carbohydrate and lipid oxidation rates. The amount of glucose taken up or oxidized during the 3 hours of the experiment was estimated by the determination of the areas under the curves for glucose uptake and oxidation rates, respectively. For the measurement of glucose uptake and oxidation by the skeletal muscle in the forearm, it was considered that muscle blood flow is equal to (0.47 × total forearm flow + 0.83) (16) and that muscle occupies 72 % of the forearm volume in normal male subjects (17).

Results are reported as the mean ± SEM. To test statistically the significance of the response of a variable (e.g., glucose uptake rate) to the different glucose loads, analysis of variance was performed for the entire set of observations in each study. To compare the differences between the mean values at each time point of the studies, the paired Student t-test was used (18).

RESULTS

Forearm blood flow did not change significantly throughout the studies (Table 1) and its average values did not differ significantly between the two oral glucose tests (G.50 = 2.62 ± 0.18 vs G.100 = 2.84 ± 0.20 ml/100 ml forearm. min). Plasma arterial (A) and venous (V) glucose levels are shown in Fig. 1. The arterial and venous plasma concentrations of glucose reached during the 100 g oral glucose study (G.100) were always higher than during the 50 g oral glucose study (G.50). The arteriovenous differences in plasma glucose were also higher in G.100 than in G.50, with statistical significance (P < 0.05) at 120 and 180 minutes. The basal forearm glucose uptake rate (Fig. 1) of the normal volunteers was similar in G.50 and G.100 studies (0.164 ± 0.033 vs 0.124 ± 0.023 mg/100 ml forearm. min, respectively). After ingestion of either glucose load, the subjects showed a significant increase in the rate of forearm glucose uptake, but this reached higher levels in response to the 100 g glucose challenge compared to the 50 g glucose challenge, in particular at 120 min (G.50 = 0.337 ± 0.097 vs G.100 = 0.920 ± 0.160 mg/100 ml forearm.min, P < 0.005) and at 180 min (G.50 = 0.073 ±

TABLE I. — *Forearm blood flow, O_2 consumption, CO_2 production, respiratory quotient (RQ), glucose oxidation rate and lipid oxidation rate in normal subjects at the postabsorptive state (0 time) and for 3 h after ingestion of 50 g (G.50) or 100 g (G.100) of glucose.*

Time (minutes)		0	30	60	120	180
Blood Flow (ml/100 ml forearm.min)	G.50	2.63 ± 0.35	2.50 ± 0.32	2.43 ±0.30	2.85 ±0.35	2.67 ± 0.37
	G.100	2.84 ±0.39	2.56 ± 0.41	2.79 ± 0.45	2.90 ± 0.46	3.10 ± 0.50
O_2 consumption (ml/100 ml forearm.min)	G.50	0.260 ± 0.04	0.279 ± 0.05	0.270 ±0.04	0.293 ±0.04	0.295 ± 0.04
	G.100	0.310 ±0.05	0.315 ± 0.06	0.337 ± 0.07	0.365 ± 0.06	0.372 ± 0.06
CO_2 production (ml/100 ml forearm.min)	G.50	0.179 ± 0.03	0.210 ± 0.04	0.192 ±0.03	0.235 ±0.03	0.262 ± 0.04
	G.100	0.212 ±0.04	0.242 ± 0.05	0.253 ± 0.05	0.312 ± 0.05	0.318 ± 0.05
Respiratory Quotient (RQ)	G.50	0.69 ± 0.02	0.75 ± 0.03	0.71 ±0.04	0.80 ±0.03	0.89 ± 0.04
	G.100	0.68 ±0.03	0.76 ± 0.03	0.75 ± 0.04	0.86 ± 0.06	0.86 ± 0.06
Glucose oxidation rate (mg/100 ml forearm.min)	G.50	0.013 ± 0.01	0.081 ± 0.04	0.043 ±0.02	0.117 ±0.04	0.207 ± 0.05
	G.100	0.015 ±0.01	0.090 ± 0.04	0.081 ± 0.03	0.195 ± 0.05	0.167 ± 0.06
Lipid oxidation rate (mg/100 ml forearm.min)	G.50	0.124 ± 0.02	0.105 ± 0.02	0.116 ±0.02	0.097 ±0.02	0.061 ± 0.02
	G.100	0.147 ±0.02	0.119 ± 0.02	0.134 ± 0.03	0.101 ± 0.03	0.115 ± 0.03
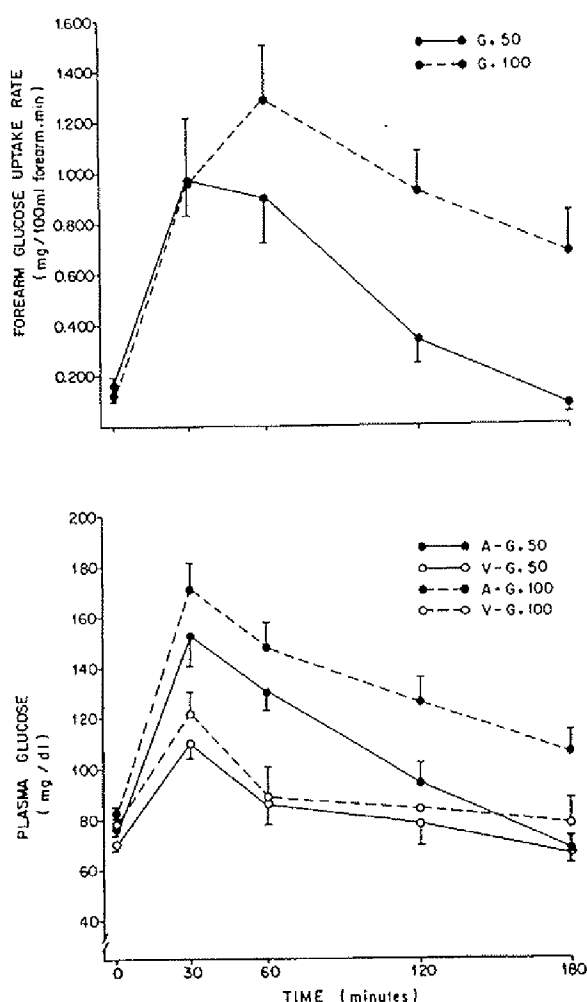
0.031 vs G.100 = 0.674 ± 0.164 mg/100 ml forearm.min, P < 0.005).

In the postabsorptive state (0 time), there were no significant differences between the G.50 and G.100 studies with respect to O_2 consumption, CO_2 production, RQ, or oxidation rates of glucose and lipids in the forearm of the participants *(Table I)*. Glucose ingestion (50 g or 100 g) was associated with a significant increase in CO_2 production and RQ, whereas the increase in O_2 consumption was only slight, reaching its highest levels during the last two hours of the studies. The rates of glucose and lipid oxidation, in accordance with the RQ values, showed that there was a clear predominance of lipid oxidation in the postabsorptive state in both tests. However, after glucose ingestion, the rate of lipid oxidation fell significantly while the glucose oxidation rate increased markedly in the forearm of the subjects, reaching levels slightly higher in the G.100 study than in the G.50 data but the difference was statistically significant at 120 minutes only (P < 0.05).

Table II shows the overall muscle glucose disposal data during the 3 hours of study. The amounts (total and above basal values) of glucose taken up by the forearm muscle of the subjects during the tests showed significant differences between loads (P < 0.005), i.e. greater in the G.100 test than in G.50 test. Extrapolating these data to total muscular tissue of the body (40 % body weight) it was evidenced that 27.0 ± 4.0 g (G.50) and 46.7 ± 4.6 g (G.100) of glucose were taken up by peripheral muscles, corresponding

FIG. 1. — *Plasma glucose levels (A : arterial; V : venous) and forearm glucose uptake rates in normal subjects at the postabsorptive state (0 time) and during 3 hours following ingestion of 50 (G.50) or 100 g (G.100) of glucose.*

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TABLE II. — *Peripheral muscle glucose disposal data (total = T and above basal values = Ab) for the 3 hours following ingestion of 50 g (G.50) or 100 g (G.100) of glucose.*

		G.50	G.100
Uptake	T	103.5 ± 16.6	178.9 ± 19.7
(mg/100 g muscle)	Ab	72.1 ± 15.1	154.3 ± 17.5
Oxidation	T	18.3 ± 5.2	23.6 ± 4.5
(mg/100 g muscle)	Ab	15.6 ± 4.8	20.5 ± 4.2
Nonoxidative metabolism (mg/100 g muscle)	T	85.2 ± 16.7	155.3 ± 23.0

respectively to 54 % and 46.7 % of the ingested loads. During the 3 hours of study, forearm glucose oxidation (total and above basal), although slightly higher in the G.100 test, was not significantly different between the two glucose challenges. On the basis of forearm glucose uptake and oxidation date, nonoxidative glucose metabolism was significantly greater during the G.100 than during the G.50 test ($P < 0.005$).

Under basal conditions (0 time), serum FFA concentrations were elevated in all subjects *(Fig. 2)*, but similar in both studies (G.50 or G.100). After glucose ingestion, serum FFA levels declined markedly in both studies to similar concentrations at 30, 60 and 120 min. During the last hour of observation, the serum FFA levels were kept suppressed in the G.100 test, while in the G.50 test they tended to increase at 180 minutes.

The serum insulin levels were similar before the ingestion of either glucose load bu the response to the challenges was significantly greater in the G.100 compared to G.50 test ($P < 0.05$).

DISCUSSION

At the cellular level in the muscular tissue, glucose disposal is dependent on its uptake and metabolism by both oxidative and nonoxidative pathways. The present study demonstrated a dose-dependent pattern of response of glucose uptake in peripheral muscle when different amounts (50 or 100 g) of glucose were administered. The increment in the forearm glucose uptake rate *(Fig. 1)* and the amounts of glucose *(Table II)* taken up by the forearm muscle of the normal subjects during the G.100 study were significantly greater than during the G.50 study. It is important to note that the higher rate of glucose uptake in forearm muscle following the ingestion of the 100 g glucose load was directly related to the greater supply of this hexose and to the adequate insulinization of this tissue (arterial glycemic and insulin levels during the last 2 hours in G.100 —
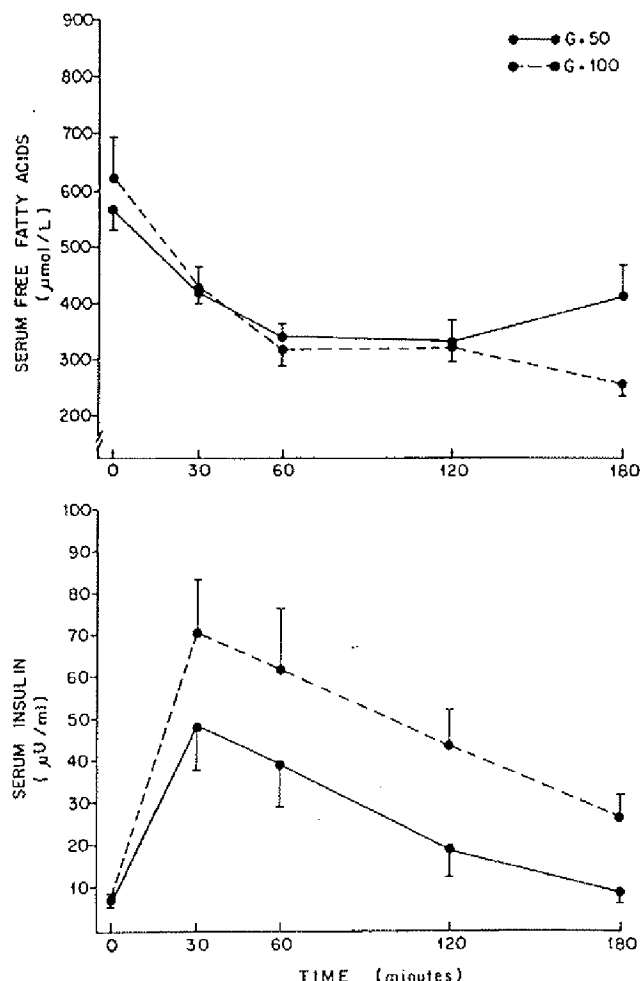
FIG. 2. — *Serum insulin and free fatty acids levels in normal subjects at the postabsorptive state (0 time) and during 3 hours following ingestion of 50 (G.50) or 100 g (G.100) of glucose.*

Fig. 1), while during the G.50 study the glucose uptake rate as well as the plasma glucose and insulin concentrations returned to levels similar to baseline values as soon as 120 minutes.

The relative importance of each organ or tissue in the disposal of an oral glucose load is still controversial (1-5, 19-21). In the present study, we observed that during the 3 hours following the ingestion fo 50 or 100 g glucose, the glucose uptake by the skeletal muscle of the whole body accounted for 27 ± 4 g (G.50) and 47 ± 5 g (G.100), comprising 54 % and 47 % of the ingested loads, respectively. This important participation of the muscular tissue in the disposal of an oral glucose load, equivalent to or even greater than the contribution of the splanchnic area, are in accordance with other observations (2-5, 21). But

more importantly, we observed that this participation of the muscular tissue in that metabolic adaptation seems to be relatively more significant with the lower dose of glucose (50 g) than with oral glucose loads of 75 g (5) or 100 g. These data suggest that the hepatic glucose uptake may play a greater contribution to the disposal of greater glucose loads with higher glycaemic levels, because this tissue contains glucokinase, a phosphorylation enzyme of high Km, while the hexokinase, with a lower Km, is the enzyme mainly responsible for glucose phosphorylation in the muscle cell.

In previous studies on fuel oxidation, in the whole human body as measured by indirect calorimetry, we (22) and Moeri *et al.* (23) observed a dose-dependent metabolic response of normal subjects to oral glucose loads of 50 and 100 g, suggesting that the modulation of this oxidative response to glucose ingestion is related to the participation of insulin-dependent tissues (mainly liver and muscle). Other studies (24-26) using the same method have already demonstrated that the oxidative pathway is more rapidly saturated than the nonoxidative metabolism of glucose when the tissue uptake of this substrate is stimulated by hyperinsulinemia and/or hyperglycemia. In the present study, the quantitation of the muscular tissue participation in this glucose cellular processing showed only a slightly higher forearm glucose oxidation in the G.100 study compared to that in G.50 study, but was not statistically significant *(Table II).* These data revealed a saturation of the muscular pathway of glucose oxidation in response to the smaller dose of glucose (50 g), and suggest furthermore that the modulation of the whole body oxidative response to different oral glucose loads seems to be mainly due to the participation of the splanchnic tissues.

The glucose disposal data for the 3 hours of study *(Table II)* also revealed that the nonoxidative metabolism of glucose represented the major metabolic fate of the glucose taken up by the muscle cells (82 % in G.50 and 87 % in G.100) while a minor portion (18 % and 13 %, respectively) was consumed by cellular oxidation. The absolute amounts of glucose metabolized through the nonoxidative pathways, predominantly stored as muscle glycogen, were also directly related to the size of the ingested glucose load.

In conclusion, this study revealed a dose-dependent metabolic response in the muscular tissue of normal subjects to oral glucose loads of 50 and 100 g, with respect to forearm muscle glucose uptake and nonoxidative glucose metabolism. The oxidative responses of the muscle tissue to the oral glucose challenges seem not to be directly proportional to the ingested meals.

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Metabolic Effects of Glucose, Mannose, Galactose, and Fructose in Man*

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ABSTRACT. The effects of various hexoses upon immunoreactive insulin (IRI) secretion, glucose disposal, and gastric inhibitory polypeptide (GIP) release have been compared in 10 normal nonobese men. Rapid iv infusion (0.5 g/kg in 3 min) of D-mannose resulted in significant IRI release, the peak levels approaching those after D-glucose infusion. D-Galactose, however, was ineffective. The 60-min urine excretions of mannose, galactose, and glucose were $35 \pm 7\%$, $16 \pm 4\%$, and $5.5 \pm 0.7\%$ (mean $\pm$ SEM) of the administered dose, respectively.

All subjects also received 50 g oral glucose, mannose, galactose, and fructose on different days, each followed by an iv glucose infusion 30 min later. The ingestion of glucose or galactose resulted in a similar increment of GIP ($P < 0.01$), followed by a similar increment in the IRI response to iv glucose. Furthermore, the glucose disposal rate increased 2.5-fold compared

to that after iv glucose alone ($P < 0.001$). However, oral mannose or oral fructose caused no significant GIP release, yet the IRI response to a subsequent iv glucose load was moderately augmented after oral mannose or oral fructose when compared to iv glucose alone. In addition, there was a similar enhancement of glucose disposal of the iv glucose load after both oral mannose and oral fructose ($P < 0.01$).

From these studies we conclude that 1) galactose does not elicit IRI secretion *per se*, yet, like glucose, potentiates GIP and IRI secretion; 2) mannose, despite weak transport across gut or kidney, evokes significant betacytotropic effects; and 3) mannose- and fructose-induced enhancement of glucose disposal might be mediated by a factor(s) other than GIP. (*J Clin Endocrinol Metab* **49**: 616, 1979)

THE UTILIZATION of nonglucose hexoses and their metabolic effects, particularly related to insulin secretion, has not been well studied in man. Several *in vitro* studies in experimental animals employing D-glucose epimers have shown that pancreatic islets oxidize D-mannose and generate ATP similar to glucose, whereas D-galactose is not metabolized (1-3). D-Fructose was found to be intermediate in these characteristics. A number of other similarities in the metabolism of D-glucose and D-mannose have been reported, including their comparable utilization by the brain to overcome hypoglycemia (4-7), by adipose tissue (8), and by the liver for glycogen synthesis (9). In contrast, mannose is poorly transported across the intestine and has a low or absent renal threshold (10). In several *in vitro* and *in vivo* experimental models, direct evidence for an insulin-re-

leasing effect of mannose has been described, wherea the nonmetabolizable epimer galactose has been show not to stimulate insulin secretion in most studies (11-15 with some exceptions (16, 17). Another hexose, D-fruc tose, on the other hand, has been found to have no direc stimulating effect on insulin secretion but potentiate insulin release in response to D-glucose (13) or D-mannos (18).

In man, effects of nonglucose hexoses on insulin secre tion have been inadequately studied with controversia results. Employing bioassay techniques for insulin assay Sheps *et al.* (19) were unable to measure significan insulin release in response to mannose. However, Karan *et al.* (20) demonstrated changes of serum immunoreac tive insulin (IRI) comparable to those seen after glucose in four out of five obese nondiabetic subjects after man nose infusion but not after galactose infusion. The effect in nonobese control subjects were not examined.

Much less is known about the effects of orally admin istered nonglucose hexoses upon the secretion of gut factor(s). Recently, considerable evidence has suggested an insulinotropic role of gastric inhibitory polypeptide (GIP) as a mediator of insulin secretion (21-23) and the

secretion of GIP in response to oral glucose seems well established (21, 22). In recent reports, galactose was shown to stimulate GIP release in normal subjects (24, 25), but the effects in response to other hexoses were not studied.

In this study, we have compared the effects of the iv infusion of various glucose epimers upon the dynamics of insulin and GIP secretion. In addition, the influences of the oral administration of glucose, mannose, galactose, and fructose on the secretion of GIP and glucose disposal in normal man has been evaluated. The preliminary results of these studies have been reported earlier (26, 27).

Materials and Methods

Ten healthy volunteers with no personal or family history of diabetes or disorders affecting carbohydrate metabolism were studied. All subjects were nonobese men, between 18–29 yr of age (mean ± SEM, 23 ± 1.2) and between 94–111% (101 ± 2.4) of desirable body weight (Metropolitan Life Insurance Tables, 1959). All studies were performed in the overnight postabsorptive state in the Clinical Research Area of the Joslin Research Laboratory starting between 0800–0900 h after 3 days of an eucaloric diet containing no less than 250 g carbohydrate/day.

Each subject underwent a 100-g oral glucose tolerance test to ensure normal glucose tolerance and evaluate insulin secretion. Subsequently, the following seven studies were performed in each subject with a minimum duration of 48 h and maximum duration of 6 weeks between two successive tests.

Hexose infusion studies

Three studies were performed utilizing the rapid iv infusion of D-glucose, D-mannose, and D-galactose. D-Glucose was administered as a standard iv glucose tolerance test (IVGTT; 0.5 g/kg over 2–4 min), as described previously (28). The same procedure was used for mannose and galactose. These hexoses were purchased from Sigma Chemical Co. (St. Louis, MO), and after sterilization, the solutions were shown to be bacteria free and pyrogen free by appropriate testing (Leberco Laboratories, Roselle Park, NJ). The glucose content of mannose and galactose was <0.005% and <0.0015%, respectively, with a yield, therefore, of greater than 99.995% purity of the glucose epimers. Blood samples were obtained for the determination of true plasma glucose (PG) concentrations [Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA) employing glucose oxidase], total plasma hexose concentrations [according to the ferricyanide method of Hoffman (29) as modified for the AutoAnalyzer], serum IRI [by a double antibody technique (30)], and serum immunoreactive GIP [by the method of Kuzio et al. (31)]. The between-assay variability for GIP in this assay was 16.9%. In addition, urine samples were collected before beginning the hexose infusion and 60 min after the infusion for the determination of true glucose and true hexose content. The urine hexose content was determined by the quantitative urine sugar technique described by Sumner (32). The plasma and urine hexose concentrations were determined by subtracting true glucose values from the total hexose levels.

Oral hexose plus glucose infusion studies

Four additional studies were performed on each subject on different days employing 50 g oral D-glucose, D-mannose, D-galactose, and D-fructose. For each test, the hexose was administered in a volume of approximately 150 ml and blood samples for true PG, total plasma hexose, IRI, and GIP were obtained at 0, 5, 10, 20, and 30 min. Immediately after the 30 min sample, an iv glucose (IV-G) infusion was administered as described for the IVGTT above, and samples were then obtained for the subsequent 60 min as previously described (28). The 30 min value for each parameter after the oral hexose load was taken as the baseline value for the IV-G infusion in each of these studies.

Calculations and statistical analyses

The PG, plasma mannose (PM), and plasma galactose (PGal) disappearance rates (K) for the hexose infusion studies were calculated by the method of Amatuzio et al. (33, 34), i.e. after subtracting the fasting hexose level (0 in the cases of mannose and galactose) from the total concentration achieved at each time interval. The same method of calculating plasma decremental glucose disappearance rate was used for all IV-G infusion studies after each oral hexose administration. This method of calculating disappearance rates was considered more appropriate since the K rates are different by this method (33, 34) which employs glucose excess above baseline rather than by the standard method (28) which employs actual glucose concentrations. Since the baseline levels of mannose and galactose in man are undetectable, the calculation of the glucose disappearance rate by this method should be the most valid comparison of disappearance rates of glucose after administration of other nonglucose hexoses.

The aggregate responses of IRI secretion after IV-G alone and for IV-G after oral hexoses were obtained by calculating the early (0–10 min) and total (0–60 min) IRI areas above the baseline. The linear regression equations of serum IRI upon PG were computed for each IVGTT and the slope of the regression line during each test was determined (35). Pooled regression coefficients (microunits per ml IRI per mg/dl PG) for the IVGTT after each oral hexose were compared with that obtained after IVGTT alone. All statistical analyses were carried out by the paired t test. All results are expressed as the mean ± SEM.

Results

Plasma hexose and IRI responses after iv glucose (IV-G), mannose (IV-M), and galactose (IV-Gal; Figs. 1 and 2)

Figure 1 presents the PG, PM, and PGal responses after various hexoses. After IV-G administration, PG rose from the fasting level of 82 ± 1.3 mg/dl, and then declined with a rate of decremental disappearance of 4.22 ± 0.85%/
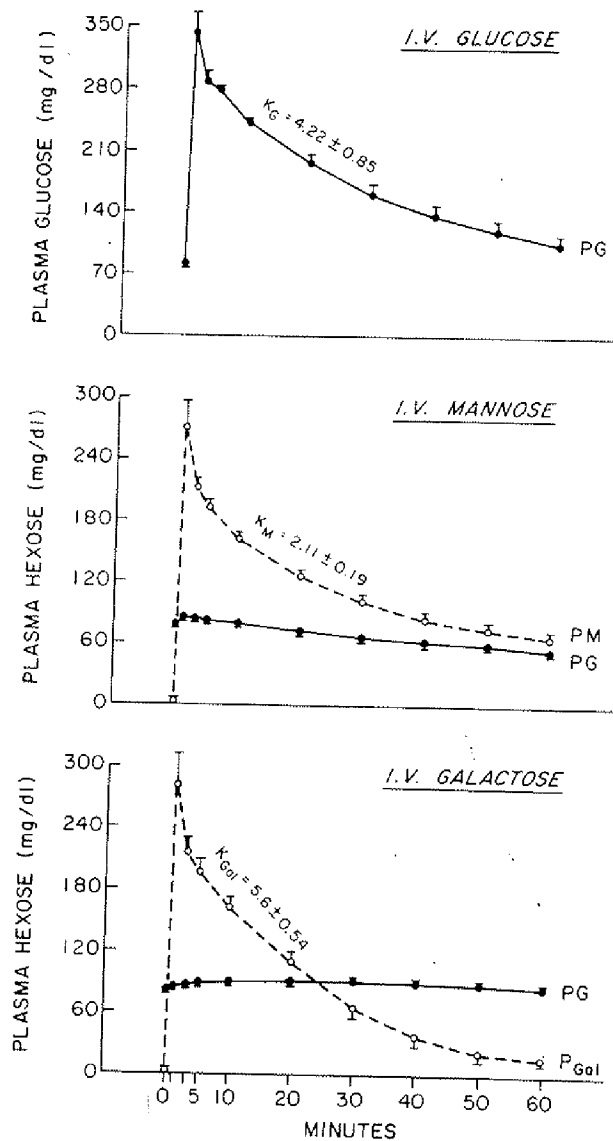
FIG. 1. PG, PM, PGal, and serum IRI concentrations after rapid iv hexose infusion (0.5 g/kg). K_G, K_M, and K_Gal represent decremental disappearance rates of glucose, mannose, and galactose, respectively. See text for details.

min. Commensurate with the PG levels, IRI rose from baseline level of 8.0 ± 0.9 to 87 ± 5.6 $\mu U/ml$ at 1 min (Fig. 2), a typical response for normal individuals (36), and declined towards baseline by 60 min. After IV-M, PG levels did not rise and, in fact, gradually declined to a mean level (57 ± 3.0 mg/dl) at 60 min that was 28% below ($P < 0.01$) the baseline, a response seen in earlier studies after IV-M (10). The PM levels, however, rose as expected and then declined, with a disappearance rate of $2.11 \pm 0.19\%/min$. The IRI rose from a basal level of 9.0 ± 1.9 to a peak level of 60 ± 10.8 $\mu U/ml$ 1 min after IV-M, similar to the response after IV-G but significantly

lower at each time period (Fig. 2). After IV-Gal to a level of 94 ± 3.9 mg/dl at 20 min that was l the basal ($P < 0.01$), with a return toward basel min. The galactose disappearance rate was 5.6 min. In striking contrast to glucose and mannose levels after IV-Gal rose very sluggishly to a pea only 14.4 ± 1.3 $\mu U/ml$ at 5 min after galactose basal level of 7.4 ± 1.0 $\mu U/ml$.

The peak IRI levels achieved after IV-G a were 6-fold and 4-fold greater, respectively, wh pared to those achieved after IV-Gal (Fig. 2).

Urine loss of hexoses (Table 1)

After IV-G administration, only 5.5% ($\pm 0.72\%$ glucose dose appeared in the urine in 60 min. In (

(Mean ± SEM; n = 10)
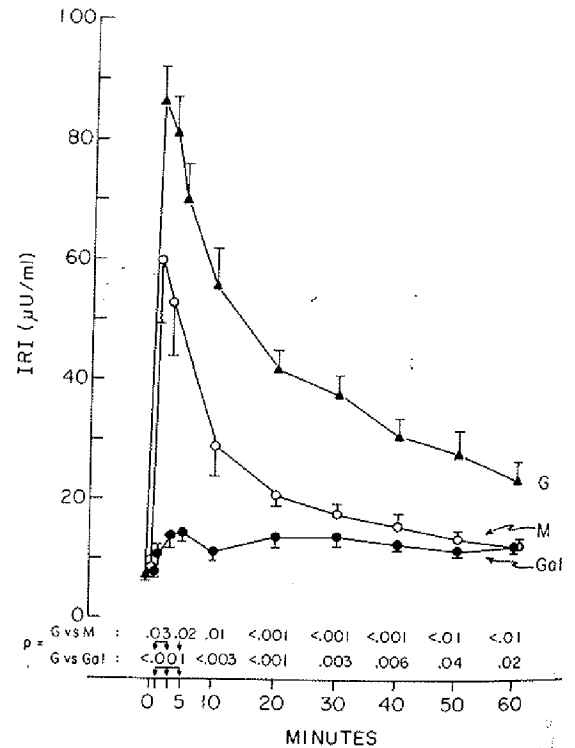
FIG. 2. Serum IRI levels after a rapid iv glucose (G), mannose galactose (Gal) infusion (each 0.5 g/kg).

TABLE 1. Comparison of urine loss of glucose, mannose, and ga during the 60 min after iv infusion of the respective hexoses

| | n | % Administered dose | | |
		Mean	SEM	P
IV-G	5	5.5	0.72	
IV-M	4	35.0	7.3	<0.01[a] <0.0
IV-Gal	4	15.7	4.5	<0.05[b]

[a] *Versus* IV-G.
[b] *Versus* IV-Gal.

more than one third of the administered dose of mannose (35.0 ± 7.3%) was recovered in the urine ($P < 0.01$ vs. glucose). The amount of galactose lost (15.7 ± 4.5%) was greater than that of glucose ($P < 0.05$) but less than that of mannose ($P < 0.05$).

Dynamics of plasma hexoses, IRI, and glucose utilization after oral hexoses

The peak plasma hexose levels occurred at 30 min and were as follows: after oral mannose, 6.5 ± 1.0; galactose, 29.0 ± 6.1; and fructose, 8.7 ± 1.8 mg/dl. No significant levels of mannose were detectable in plasma.

Figure 3 presents the PG and IRI concentrations during those studies in which various oral hexoses were administered, followed 30 min later by IV-G. After oral glucose, PG rose quickly, with peak levels at 30 min. After oral galactose or fructose, there was a modest but significant rise of PG, the mean increments from basal at
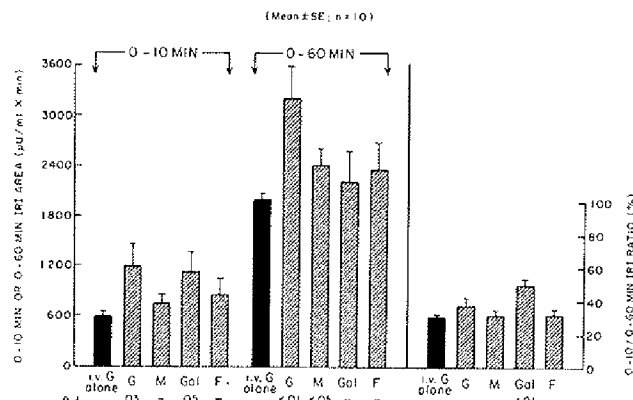
FIG. 4. Early (0–10 min) and total (0–60 min) insulin response to IV-G (0.5 g/kg) after oral glucose (G), mannose (M), galactose (Gal), or fructose (F). See text for details.

30 min being 10 ± 1.5 and 8.0 ± 2.2 mg/dl, respectively. However, after oral mannose, no detectable rise in PG occurred.

The responses of PG levels to IV-G infusion 30 min after each oral hexose administration are shown in Fig. 3 compared with PG levels after IV-G alone. The concentrations of PG were maximum at 30 min after oral glucose; the PG levels rose much higher in this group and least in the oral mannose study. The decremental rates of disappearance of glucose were compared with IV-G alone, where no oral hexose preceded the infusion. The mean rates for IV-G after oral glucose and oral galactose were comparable (10.1 ± 1.2% and 10.7 ± 1.3%/min, respectively) and more than 2.5-fold greater than that after IV-G alone (4.2 ± 0.85; $P < 0.001$ in each case). The mean rate for IV-G after oral mannose was also significantly greater (7.2 ± 1.3, $P < 0.01$) compared to that after IV-G alone. Similarly, the rate for IV-G after oral fructose was also greater (6.8 ± 0.96; $P < 0.01$) compared with IV-G alone and was nearly identical to that for the oral mannose study (p = NS).

Figure 3 also presents the IRI responses at each time interval for the oral hexose studies. After oral glucose, galactose, and fructose, there were significant increments of IRI levels, the peak levels at 30 min being approximately 7.5-, 4-, and 2.5-fold greater, respectively, than the basal levels. After oral mannose, there was no significant increment of IRI levels up to the 30-min interval. The IRI responses to IV-G administration 30 min after each of the four oral hexose studies were analyzed as the increments above the basal (30 min) levels, as shown in Fig. 4. The 0–10 min, 0–60 min, and 0–10 to 0–60 min IRI area ratios were arbitrarily selected to evaluate early insulin response, total insulin response, and proportion of total insulin response appearing rapidly after the perturbation. These are shown in comparison to the same indices obtained from IRI levels after IV-G alone. The 0–
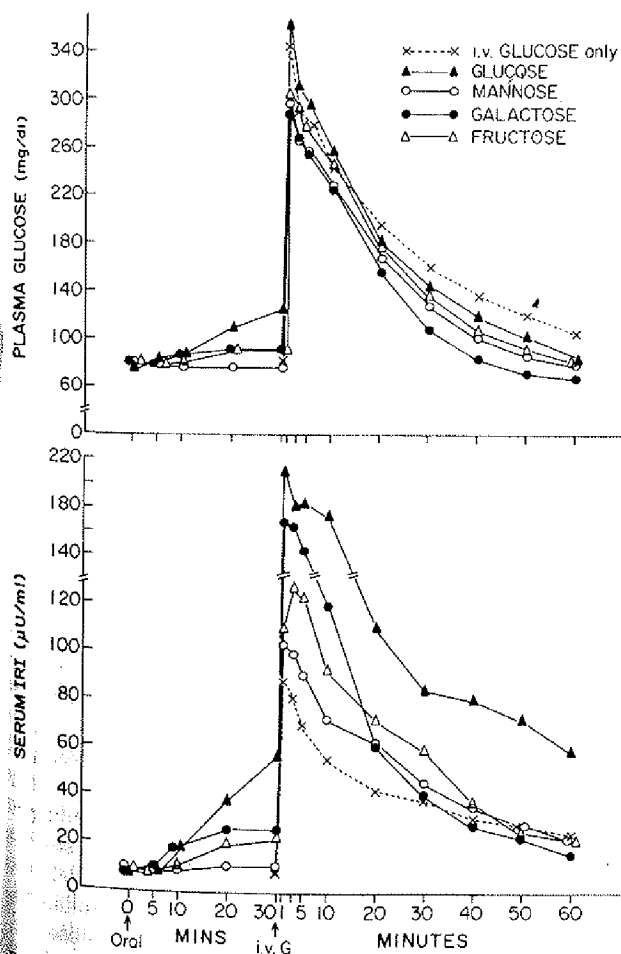
FIG. 3. PG and serum IRI concentrations after 50 g oral glucose (G), mannose (M), galactose (Gal), or fructose (F) and after a rapid iv glucose infusion (0.5 g/kg) 30 min after oral G, M, Gal, or F.

10 min IRI areas for IV-G after oral glucose ($P < 0.03$) and oral galactose ($P < 0.05$) were significantly greater than that for IV-G alone. The modest increases in the cases of oral mannose and fructose were not significantly greater than that of IV-G alone. In contrast, the 0–60 min IRI areas were significantly greater for IV-G after oral glucose and mannose than for IV-G alone but did not achieve statistical significance for IV-G after oral galactose or fructose. The augmentation of early IRI response by oral galactose was reflected by an increased 0–10 to 0–60 min IRI area ratio for IV-G after oral galactose ($P < 0.01$).

To compare further the aggregate IRI secretory responses during IV-G after oral hexoses, pooled regression coefficients (b) for each type of test, generated from the individual regression equations of serum IRI upon PG for each test, were examined and compared. The regression coefficients for each IV-G study after each oral hexose administration are shown in Table 2. The mean regression coefficient for IV-G after oral galactose (0.570 ± 0.076) was the greatest when compared to that for IV-G alone ($P < 0.001$), followed by oral glucose ($P < 0.05$), oral mannose ($P < 0.05$), and oral fructose. However, there was no significant difference between the mean b value for the oral glucose study compared to the oral galactose study. The mean b value for the oral fructose study was not significantly greater than that for IV-G alone, clearly due to greater variability in IRI responses for IV-G after oral fructose (Table 2).

Immunoreactive GIP levels after oral hexoses

Figure 5 presents the mean changes of the serum GIP concentrations in each of the four oral hexose studies expressed as the percent change from fasting levels. Oral glucose and galactose resulted in significant and similar increments in the serum GIP percent change within 30 min, whereas no significant changes in GIP occurred after oral mannose or fructose. The actual mean concentrations of GIP in the fasting state and the peak levels achieved after the various oral hexoses were as follows. After oral glucose, the levels of GIP increased from 161 ± 31 to 387 ± 67 pg/ml ($P = 0.005$). Similarly, oral

(Mean Levels; n = 8)
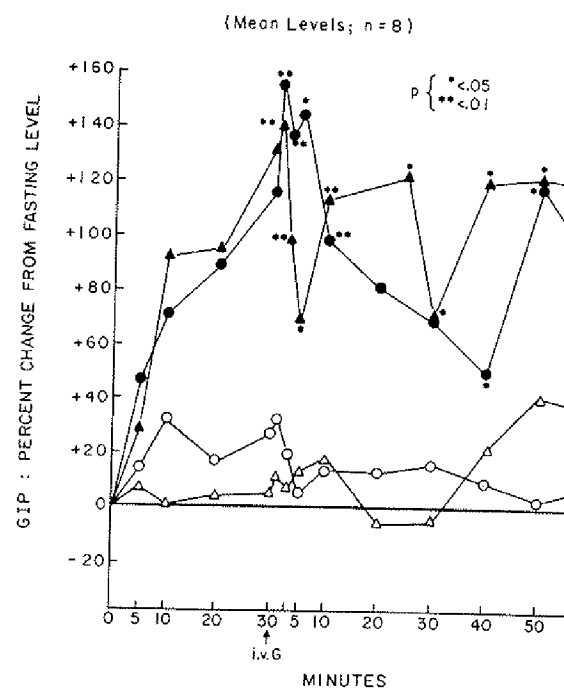
FIG. 5. GIP concentrations (percent change from fasting leve 50 g oral glucose (G), mannose (M), galactose (Gal), or fructose

galactose resulted in a significant rise from 191 ± 420 ± 51 pg/ml ($P < 0.02$). However, the mean inc in GIP after oral mannose (from 221 ± 67 to 237 and oral fructose (from 152 ± 25 to 168 ± 24) we: significant. The GIP response to the oral hexose pressed as the GIP area above the baseline, di correlate significantly with the IRI areas after the sequent IVGTT or with the respective glucose decr tal disappearance rates.

Discussion

Our studies of the effect of iv infusions of va glucose epimers clearly indicate that D-mannose evoke a significant insulin secretory response of th man pancreas. On the basis of these experimen normal men and similar results in an earlier stud obese individuals (20), it appears that the islet subs utilization characteristics for glucose and mannose correlate with their capacity to stimulate insulin rel The nonmetabolizable epimer, galactose, however not provoke an appreciable insulin secretion, a findi agreement with most of the animal experimental stu *in vivo* and *in vitro* (11–15). The sluggish and dela IRI rise seen after IV-Gal was most likely secondar its conversion to glucose in liver and kidney, as obsei in other studies (37). In regard to mannose, it interest that there was a significant hypoglycemic ef with a mean PG decline of 28% below the basal le

TABLE 2. Pooled regression coefficients (b) for serum IRI upon PG for IV-G after each oral hexose

Perturbation	μU/ml per mg/dl (mean ± SEM)	P
IV-G alone	0.249 ± 0.019	
IV-G after G	0.429 ± 0.073	<0.05,[a] NS[b]
IV-G after M	0.331 ± 0.033	<0.05[a]
IV-G after Gal	0.570 ± 0.070	<0.001,[a] <0.01[b]
IV-G after F	0.354 ± 0.078	NS[a]

[a] *Versus* IV-G alone.
[b] *Versus* IV-G after M.

during the 60 min after its infusion, most likely due to competition of mannose-6-phosphate with glucose-6-phosphate for glucose-6-phosphatase (10). In addition, a further inhibitory effect upon hepatic glucose output as a result of the augmented insulin release might contribute to the hypoglycemic effect of mannose infusion. None of the subjects studied, however, had clinical symptoms of hypoglycemia. This was not surprising since mannose has been shown to be an excellent substrate for brain tissue (4–7). After oral mannose, none was detected in the plasma and as much as 35% of the iv administered dose was recovered in the urine 1 h after infusion, findings consistent with earlier observations that the transport of mannose across gut and kidney occurs by passive diffusion rather than active transport (10). It would appear, therefore, that spatial configuration of the carbon-2 is specific for transport by gut and kidney but is not specific for hexose metabolism, islet substrate utilization, or insulin release.

The experiments designed to study the effects of various oral hexoses on the utilization of a subsequent iv glucose load yielded several interesting features. Considerable evidence now indicates that GIP might be an important mediator of the entero-insular axis in man, and a striking release of GIP in response to oral glucose has now been convincingly demonstrated in several studies (21, 22, 25, 38). Therefore, the GIP release in response to various hexose loads and the relationship to the subsequent rate of glucose disposal after an iv glucose load were examined. The results indicated considerable discordance between GIP release and subsequent glucose disposal. Both oral glucose and oral galactose resulted in equivalent increases in GIP release (Fig. 5) and enhancements of the subsequent IV-G disposal rate. The IRI release for IV-G was also potentiated by prior ingestion of oral glucose or galactose, as indicated by the acute IRI response (Fig. 4) and the slopes of the regression lines for IRI upon PG (Table 2). On the other hand, neither oral mannose nor fructose resulted in a significant GIP release, yet both resulted in a significant augmentation of the IV-G disposal rate ($P < 0.01$). Furthermore, the IRI release for IV-G after oral mannose was greater than that for IV-G alone, as seen by the total IRI area and the mean slope of the regression line of IRI upon PG (Table 2). The mean IRI responses for IV-G after oral fructose were almost identical to those after oral mannose but did not attain statistical significance because of somewhat greater variability (Fig. 4 and Table 2). There was no significant correlation between the IRI release or the glucosal disposal rate and the GIP response for any of the oral hexose studies, although this could be due to the small number of subjects studied.

These observations indicate that oral ingestion of certain substrates, such as mannose and fructose, might

elicit release of a as yet unidentified gut factor(s) (39) which might enhance IRI release or potentiate the tissue sensitivity to endogenous IRI. In this regard, it is of interest that preliminary data indicate significant extra-pancreatic effects of GIP on adipose tissue (40) which could mediate enhanced insulin sensitivity.

Acknowledgments

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71833 SED99|DIABETES CARE.|1983 VOL 6 PT 5|PP 441-445|HEINE, RJ & HANNING,
I|THE ORAL GLUCOSE TOLERANCE TEST: EFFECT|OF RATE OF INGESTION OF
CARBOHYDRATE AND|DIFFERENT CARBOHYDRATE PREPARATIONS

The Oral Glucose Tolerance Test (OGTT): Effect of Rate of Ingestion of Carbohydrate and Different Carbohydrate Preparations

ROBERT J. HEINE, M.D., IAN HANNING, B.Sc., LINDA MORGAN, Ph.D., AND K. GEORGE M. M. ALBERTI, D.Phil.

The glucose load of the oral glucose tolerance test (OGTT) is well standardized. However, recommendations on rate of ingestion and nature of the load are vague. In this study the effect on blood glucose, serum insulin, C-peptide, and plasma gastric inhibitory polypeptide (GIP) of giving 75 g glucose in 300 ml over 1 and 10 min (G1 and G10) was investigated in six subjects. In five an isocaloric amount of partially hydrolyzed starch (Hycal) was also used (H1 and H10). The fast glucose intake, compared with the slow ingestion, resulted in an earlier rise in blood glucose levels, accompanied by a faster serum insulin and C-peptide response. Between 90 and 135 min blood glucose concentrations were significantly higher after the 10-min glucose intake. At 120 min blood glucose levels were 5.5 ± 0.5 and 4.7 ± 0.5 mmol/L, respectively, for G10 and G1 ($P < 0.05$). In the first half hour after slow and fast Hycal intake no differences were seen in blood glucose, serum insulin, and C-peptide levels. Between 45 and 120 min blood glucose levels were significantly higher after the 10-min Hycal intake. At 120 min blood glucose levels were 5.3 ± 0.2 and 4.4 ± 0.1 mmol/L, respectively, for H10 and H1 ($P < 0.01$). Except for a faster rise in glucose and insulin levels after glucose loading in 1 min, no further differences were found, when compared with Hycal. No significant differences were seen in the GIP responses. Thus differences in rates of ingestion can cause significant differences in later results. A standard time for glucose ingestion should be specified. DIABETES CARE 6: 441–445, SEPTEMBER–OCTOBER 1983.

Considerable effort has been expended on standardizing the glucose load of the oral glucose tolerance test (OGTT) and the diagnostic criteria for its interpretation.[1-5] However, recommendations on rate of ingestion and nature of the load have been variable or vague. The World Health Organization advises a glucose load of 75 g in 250–350 ml water, which should be consumed in 5–15 min[5] whereas the Australian Association of Clinical Biochemists suggests the same glucose load but ingested within 5 min.[6] The latter, in practice, often results in ingestion in 1–2 min.

To our knowledge the effect of different glucose ingestion rates on glucose and insulin levels during an OGTT has not been investigated. There is thus little evidence to support these recommendations. Other carbohydrates such as partially hydrolyzed starch or maltose have been accepted instead of glucose as the carbohydrate load for the OGTT.[4,5,7,8] The advantage of maltose and hydrolyzed starch over glucose is the less frequency of side effects such as nausea and vomiting.[7]

The aim of our study was to compare two different inges-

tion rates, one the fastest reasonable rate and the other at the midpoint of the WHO suggested range, of both a glucose and a partially hydrolyzed starch load on blood glucose, serum insulin, C-peptide, as well as on plasma gastric inhibitory polypeptide (GIP) levels, in the OGTT.

MATERIALS AND METHODS

Subjects. Six normal volunteers were recruited, three men and three women 21–30 yr old. Ideal body weight ranged from 91% to 118% (Metropolitan Life Insurance Tables, 1959). None were taking any medication or known to be suffering from any disease. All volunteers maintained their usual diet before the investigations. The studies were performed at 8:30 a.m. after an overnight fast.

Protocol. Subjects participated in a random order in the study comparing fast (in 1 min, G1) and slow (in 10 min, G10) intake of 75 g of glucose, dissolved in tap water to a volume of 300 ml. In five of these volunteers the study was repeated using an isocaloric amount of Hycal (Beecham Prod-

TABLE 1
Hycal: carbohydrate constituents

	% (wt/wt)
Anhydrous D glucose	19.4
Disaccharides	14.2
Trisaccharides	11.8
Tetrasaccharides	9.9
Pentasaccharides	8.4
Hexa- and higher saccharides	36.3

ucts, Brentford, United Kingdom) (partially hydrolyzed starch; Table 1), dissolved to a volume of 300 ml, again ingested in 1 min (H1) and 10 min (H10).

Free flowing venous blood samples were taken from an antecubital vein before and for 240 min after ingestion of the carbohydrate load. Sampling was every 5 min during the first half hour and quarter-hourly afterward. Tests were performed at least 1 wk apart. Nausea was not felt by any subject.
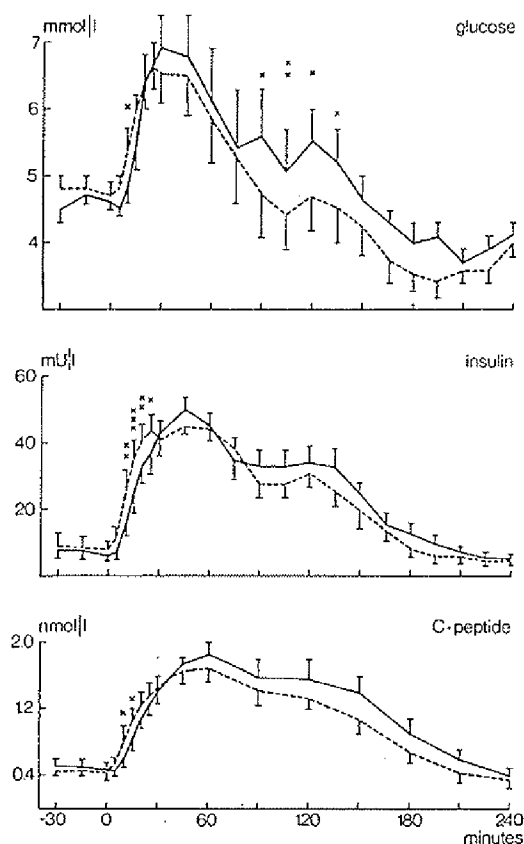
FIG. 1. Blood glucose, serum insulin, and C-peptide response to 75-g glucose loading in 1 and 10 min. G1: ---; G10:——; x: P < 0.05; xx: P < 0.02; xxx: P < 0.01.

FIG. 2. Area under blood glucose, serum insulin, and C-peptide curves from 0 to 15 and 0 to 30 min after glucose and Hycal loading in 1 and 10 min: G1, G10, H1, and H10. x: P < 0.05; xx: P < 0.02; xxx: P < 0.01.

Glucose and hormone estimations. Blood for glucose assay was immediately deproteinized in 5 ml ice-cold 5% (vol/vol) perchloric acid and estimated by a standard fluorimetric method.[9] Serum insulin was measured by double-antibody radioimmunoassay.[10] Serum C-peptide was measured by radioimmunoassay with ethanol precipitation.[11] Plasma GIP levels were measured by radioimmunoassay.[12]

Statistical analysis. Results are expressed as mean and SEM. The two-tailed Student paired t-test was used to assess sta-
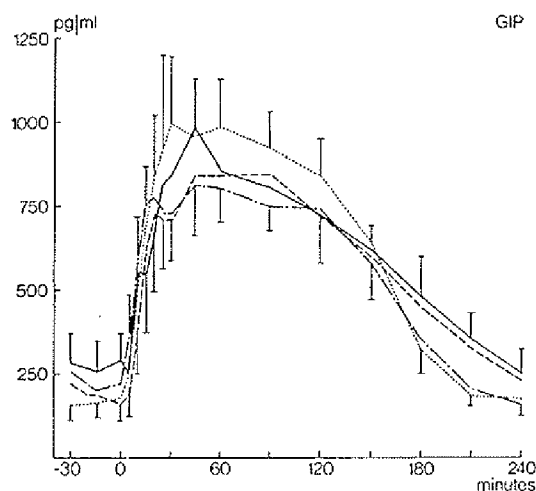
FIG. 3. Plasma GIP responses to glucose and Hycal loading in 1 and 10 min. G1: ·——·——; G10: ---; H1: ——; H10: · · ·.

tistical significance of differences. Areas under the glucose and insulin curves were calculated with the formula:

$$Area = \frac{\Sigma (X_{n+1} - X_n)(Y_n + Y_{n+1})}{2}$$

where X_n = sampling time in minutes at time point n and Y_n = concentration of measured substance at time point n.

RESULTS

Comparison between 75 g glucose ingestion in 1 and 10 min. The fast glucose intake resulted in an earlier rise in blood glucose levels (Figure 1). The area under the glucose curve between 0 and 15 min was significantly greater with G1 than G10 (6.1 ± 1.4 versus 2.6 ± 0.6 mmol/L · min, P < 0.05) (Figure 2).

This was accompanied by faster serum insulin and C-peptide response in the G1 study (Figures 1 and 2). The areas under the insulin and C-peptide curves between both 0 and 15 min and 0 and 30 min were significantly greater after G1. The insulin areas were 173 ± 39 and 71 ± 25 mU/L · min (P < 0.05) and 653 ± 99 and 469 ± 78 mU/L · min (P < 0.05) at 0–15 and 0–30 min, respectively, while the corresponding values for C-peptide were 3.8 ± 0.9 versus 1.2 ± 0.3 nmol/L · min (P < 0.02) and 16.2 ± 2.5 versus 11.1 ± 1.5 nmol/L · min (P < 0.05).

Plasma GIP levels were not significantly different with either rate of ingestion (Figure 3).

Between 90 and 135 min blood glucose concentrations were significantly higher after G10 (Figure 1). At 120 min the glucose levels were 5.5 ± 0.5 and 4.7 ± 0.5 mmol/L, respectively, for G10 and G1 (P < 0.05).

The areas under the glucose curves between 0 and 120 min and between 0 and 240 min were not significantly dif-

ferent for G1 and G10. The insulin response, however, was significantly greater after G10, calculated over 240 min: 4211 ± 771 versus 3654 ± 655 mU/L · min (P < 0.02).

Total C-peptide response (0–240) also tended to be greater after G10, but the difference did not achieve statistical significance (Figure 4). Plasma GIP responses were similar on both occasions.

Comparison between ingestion of 120 ml Hycal in 1 and 10 min. In the first 30 min no differences were seen in blood glucose, serum insulin, C-peptide, or plasma GIP responses.
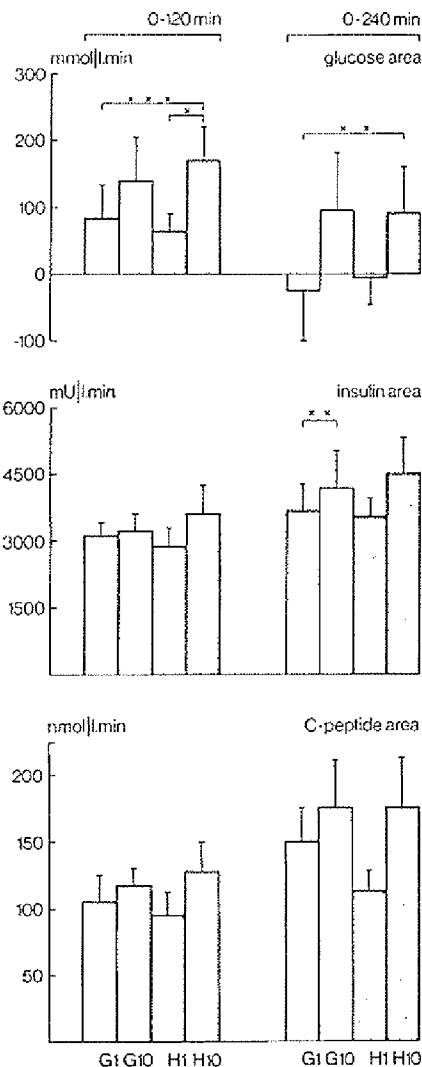
FIG. 4. Area under blood glucose, serum insulin, and C-peptide curves from 0 to 120 and 0 to 240 min after glucose and Hycal loading in 1 and 10 min: G1, G10, H1, and H10. x: P < 0.05; xx: P < 0.02; xxx: P < 0.01.
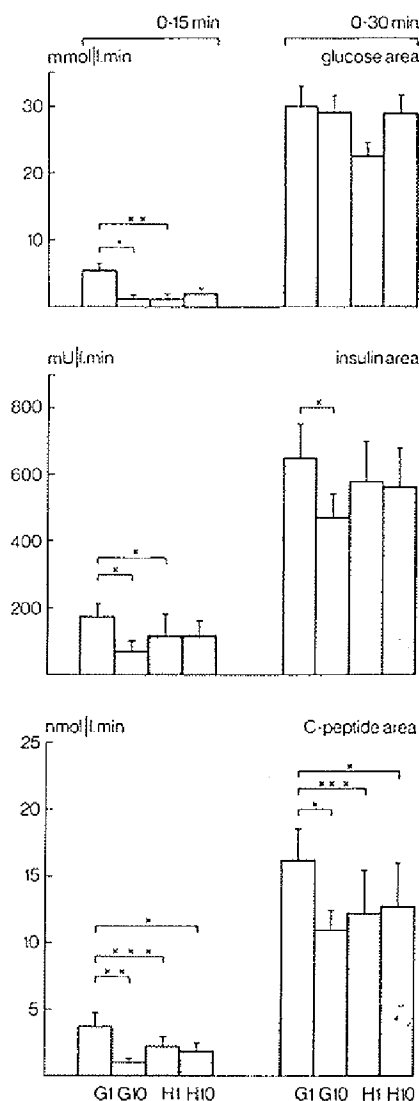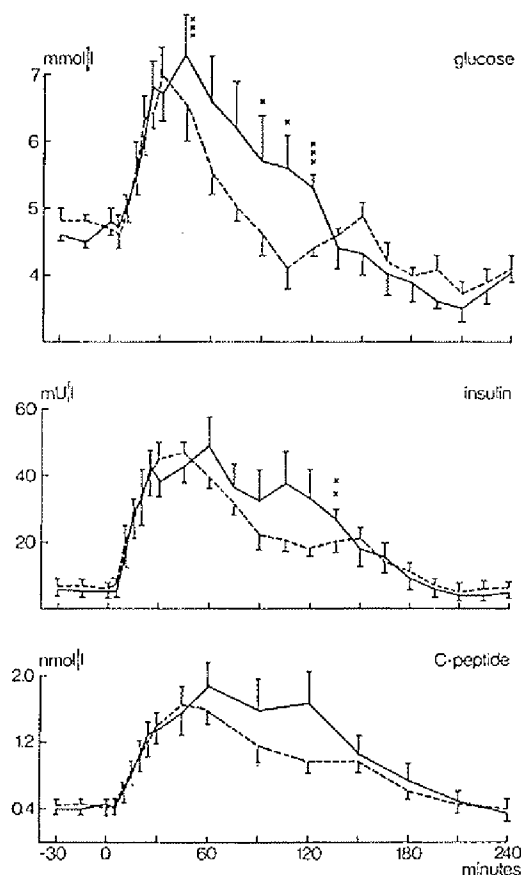
FIG. 5. *Blood glucose, serum insulin, and C-peptide response to 120 ml Hycal in 1 and 10 min. H1: ---; H10: ——. x: $P < 0.05$; xx: $P < 0.02$; xxx: $P < 0.01$.*

Between 45 and 120 min the glucose levels were significantly higher at four time points after slow Hycal intake (Figure 5). At 120 min the blood glucose levels were 5.3 ± 0.2 and 4.4 ± 0.1 mmol/L, respectively, for H10 and H1 ($P < 0.01$).

The area under the glucose curve from 0 to 120 min was significantly smaller after H1: 65.4 ± 23.8 versus 171.3 ± 51.6 mmol/L · min for H1 and H10 ($P < 0.05$) (Figure 4).

The insulin, C-peptide, and GIP responses were not significantly different, either from 0 to 120 min or from 0 to 240 min (Figures 3 and 4).

Comparison between glucose and Hycal. The area under the glucose curve between 0 and 15 min was significantly greater for G1 compared with H1 (6.1 ± 1.7 and 2.4 ± 1.6 mmol/ L · min, $P < 0.02$). No significant differences were found between G10 and H10 (Figure 2). The area under the insulin curve between 0 and 15 min was significantly greater for G1 compared with H1 (168 ± 47 and 118 ± 45 mU/L · min, respectively, $P < 0.05$). The areas under the C-peptide curves

both from 0 to 15 and 0 to 30 min were significantly greater for G1 compared with H1 ($P < 0.01$ for both). The insulin, C-peptide, and GIP responses over 120 and 240 min were not significantly different for glucose and Hycal loading.

DISCUSSION

The results of this study indicate that the rate of intake of the carbohydrate load and to a lesser degree the nature of the load influence the results of the OGTT. The earlier glucose response after fast glucose intake might be explained by the time needed for digestion of poly- and disaccharides in the intestinal lumen and cellular brush border, in contrast to the direct glucose absorption by a special carrier mechanism.[13,14] Except for the faster rise in glucose and insulin levels after glucose loading in 1 min, no further differences were found when compared with Hycal. GIP responses were similar. GIP has been recognized as an important insulinotropic hormone.[15] However, it seems only to act as such when there is slight hyperglycemia.[16]

The fast insulin response after the 1-min glucose intake seems to be unrelated to GIP but dependent entirely on the rate of glucose rise.

The 2-h glucose value, crucial now as a diagnostic criterion, was strongly influenced by the rate of intake of the carbohydrate load. The lower 2-h glucose value after the fast glucose intake might partially be explained by the earlier insulin response; however, no differences in insulin response were seen when slow and fast Hycal intake were compared. The area under the glucose curve from 0 to 120 min was significantly greater after the 10-min Hycal intake. With a similar insulin response it may be assumed that the amount of glucose absorption was greater after slow Hycal ingestion. This suggests that the fast intake of an hyperosmolar fluid, mainly consisting of polysaccharides, impairs glucose absorption, possibly by increasing the rate of intestinal transit.[13] The areas under the glucose curves after glucose intake were not significantly different, perhaps because of the faster absorption of glucose.

The 2-h glucose value is thus influenced by the rate of intake of the carbohydrate load, possibly by the early insulin response, as seen with fast glucose loading and/or by the influence on glucose absorption with fast Hycal ingestion.

Thus we have shown that the results of the OGTT can be influenced by the rate of ingestion of the carbohydrate load. We chose to compare the fastest practical rate of ingestion, 1 min, with the midpoint of the WHO suggested range, 10 min. It is possible that if 5 min had been compared with 15 min smaller differences would have been found. The effect of different rates of ingestion in diabetic subjects was also not studied. However, it seems reasonable to conclude that a standard time for the rate of ingestion of the carbohydrate load should be specified and used uniformly, both for diagnostic purposes and in epidemiologic surveys.

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Sucrose Ingestion, Insulin Response and Mineral Metabolism in Humans[1,2]

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ABSTRACT The effects of sucrose ingestion on the excretion of urinary calcium, zinc, phosphorus, sodium and potassium have been investigated and compared among 13 individuals to the magnitude of their postprandial serum insulin response. Fasted subjects consumed a beverage containing 2 g sucrose/kg, and urine and blood samples were taken at intervals during the next 3 h. As a result of sucrose consumption there were significant increases in serum insulin and decreases in serum phosphorus, but no change in serum total or filterable calcium, zinc, sodium or potassium. Urine calcium peaked at 1.5 h and was significantly increased from 1.0 through 2.5 h. Sucrose-induced increases in serum insulin and urine calcium were highly variable among subjects, and within the group were significantly correlated ($r = 0.82$, $P < 0.01$). Urine calcium excretion was correlated with serum phosphorus ($r = 0.41$, $P < 0.05$) and urine zinc ($r = 0.80$, $P < 0.01$). Sucrose consumption also increased the urinary excretion of zinc and sodium, although renal reabsorption was not impaired. The effects of sucrose on urinary calcium are consistent with the hypothesis that insulin inhibits renal calcium reabsorption. *J. Nutr.* 117: 1229–1233, 1987.

INDEXING KEY WORDS:

• sucrose • minerals • insulin • urinary excretion

The consumption of protein induces a calciuretic response within 30 min and is associated with a decrease in renal calcium reabsorption [1]. We have proposed that the inhibition of calcium reabsorption is mediated by the insulin response to protein consumption. In human subjects, the magnitude of the calciuretic response to protein is proportional to the postprandial increase in serum insulin, with approximately 30% of subjects showing little or no response in either parameter [2]. Infusion of rats with the insulin secretogogues glucose or arginine produces an increase in urine calcium that is significantly correlated with serum insulin levels. Mannoheptulose, which supresses insulin secretion, blunts the calciuretic response to glucose and arginine [3].

These data support the hypothesis that insulin is involved in the hypercalciuretic response to protein consumption. If insulin mediates this process, then carbohydrates that stimulate insulin secretion should also induce hypercalciuria. The ingestion of galactose [4], glucose [5], fructose [6], sucrose [7] and wheat starch [8] has been demonstrated to produce a calciuretic response, whereas the poorly metabolized carbohydrate xylose, and fat, do not [6]. These responses are consistent with the effect of each of the dietary constitu-

ents on insulin secretion. Also, insulin infusion in euglycemic humans increases urinary calcium excretion [9].

The present study was undertaken to determine if there is a range in the calciuretic response to sucrose ingestion among individuals, and whether the magnitude of this response is associated with postprandial serum insulin levels and renal calcium reabsorption. Because insulin infusion and carbohydrate loading have been associated with decreases in the excretion of urine sodium [9, 10] and potassium [6, 9] and both increases [6] and decreases [9, 10] in urine phosphate, changes in the excretion of these electrolytes were also investigated.

High protein meals [1], histidine [11, 12], cysteine and ethanol [13] increase urinary zinc excretion. Diabetic humans [14, 15] and streptozotocin diabetic rats [16] exhibit hyperzincuria, whereas insulin treatment of diabetic rats [16] and insulin infusion in dogs [17]

inhibit zinc excretion. Blood zinc levels have been reported to decrease after meals [18] and after ingestion of a glucose load [19]. In the present study urine and serum zinc levels were analyzed to investigate the relationship among sucrose ingestion, insulin secretion and zinc excretion.

METHODS

Thirteen adults ranging in age from 19 to 35 yr participated in this study. The average weight of the nine females was 57 kg, whereas that of the four males was 80 kg. Informed consent was obtained from each subject and the experiments were approved by the Human Investigation Committee of the University of Connecticut.

Each subject consumed a standard evening meal and fasted from 2030 h the night before the test. On awakening the next morning, subjects voided, recorded the time, drank 250 mL distilled water and reported to the laboratory. Baseline blood and urine samples were collected 15 min before the test meal was ingested. At $t = 0$ (0900 h) subjects were given a sucrose load of 2 g sucrose/kg body weight. This drink was prepared by mixing the appropriate amount of sugar with 250 mL of distilled water and 1.5 g lemon flavoring. Subjects consumed the beverage over a 5-min period.

Blood samples were drawn at 0.5, 1.0, 2.0 and 3.0 h after sucrose consumption. Urine samples were collected at 0.5-h intervals for 3.0 h postprandially. A steady diuresis was maintained by providing subjects with 200 mL distilled water every 0.5 h. Serum and urine samples were stored frozen in acid-washed containers before analysis.

Serum and urine samples were analyzed for calcium, sodium, potassium and zinc by atomic absorption spectrophotometry (Perkin-Elmer Model 2380, Norwalk, CT). Filterable calcium was determined by atomic absorption after filtering the serum samples (Ultrafree Calcium Filters, Worthington Diagnostics, Freehold, NJ). Serum insulin was determined by a double antibody radioimmunoassay (Pharmacia Diagnostics, Piscataway, NJ). Creatinine and phosphorus in serum and urine were measured by an autoanalyzer (Technicon Instruments, Tarrytown, NY). Glomerular filtration rate (GFR) was approximated by creatinine clearance. Calcium reabsorption was calculated as the difference between urine calcium and filtered calcium, where filtered calcium equals the product of GFR and serum filterable calcium. The reabsorption of phosphorus, sodium and potassium was estimated as the product of serum concentration and GFR minus urinary excretion. Zinc reabsorption was not calculated because only approximately 1% of total serum zinc is ultrafilterable, and the filterable fraction was not measured here.

Analysis of variance (ANOVA) with a random block design was used to detect changes in the parameters measured. When ANOVA indicated significant changes ($P < 0.05$), significant differences between means were determined by Scheffé's contrasts [20]. Regression analysis was performed on selected pairs of variables [20].

RESULTS

Table 1 provides a summary of the changes in GFR, serum insulin and minerals in serum and urine as a result of consuming the sucrose load. GFR was not increased at any time point. Urinary calcium excretion rose on average from 58 μg/min at baseline to a peak of 179 μg/min at 1.5 h. The rise in urine calcium excretion was also significant at $t = 1.0$ through 2.5 h. As expected, calcium excretion varied greatly among subjects. For example, individual peak excretion rates ranged from 119 to 377 μg/min. The percentage of filtered calcium reabsorbed by the kidneys decreased from a high of 99.1% at the baseline to a low of 97.3% at $t = 1.5$ h ($P < 0.001$) and as expected followed a similar but inverse pattern compared to urine calcium. The percentage of calcium reabsorbed was significantly decreased from baseline values at $t = 1.0$ through 2.5 h.

Serum insulin and serum phosphorus exhibited significant changes after sucrose consumption while serum total and filterable calcium, zinc, sodium and potassium did not. Insulin increased from an average baseline value of 11.4 to 66.5 μU/mL at $t = 0.5$ h, a rise that preceded the increase in calcium excretion by approximately 0.5 h. Insulin levels peaked at 0.5 h for approximately half of the subjects, and as was the case for urine calcium, peak values varied greatly among individuals. For example, at $t = 0.5$ h, insulin concentration ranged from a low of 26.2 μU/mL to a high of 122.8 μU/mL among different subjects.

Serum phosphorus fell significantly by $t = 1.0$ and was lowest on average at $t = 2.0$ h. This decrease followed the peak in calcium excretion by 0.5 h. Urinary phosphorus was not significantly different from baseline at any time point, but peaked at $t = 1.0$ and then fell so that the rate of excretion was significantly lower between $t = 2.0$ and $t = 3.0$ h than it was at $t = 1.0$ h. The renal reabsorption of phosphorus followed a similar but inverse pattern.

Urine zinc excretion was significantly increased from baseline levels at $t = 1.0$, 1.5 and 3.0 h. The change from 300 ng/min at $t = 0$ to 863 ng/min at $t = 3.0$ represents an increase of 190%.

Urine sodium was increased from baseline levels at $t = 3.0$, but neither serum sodium nor sodium reabsorption was altered postprandially. Among individuals, postprandial increases above baseline in urine sodium and calcium were correlated ($r = 0.57$, $P<0.05$). There was no effect of sucrose on urine potassium excretion or the percent of potassium reabsorbed.

To determine if postprandial urine calcium and serum insulin responses are associated, these two parameters

TABLE 1

Changes in glomerular filtration rate (GFR), serum insulin and minerals in serum and urine after sucrose consumption[1,2]

Measure	0 h	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h
GFR, mL/min	84.3 ± 37.1	90.3 ± 28.6	102.8 ± 35.6	98.7 ± 23.7	100.4 ± 28.6	95.4 ± 24.9	98.8 ± 23.0
Insulin, µU/mL	11.4 ± 3.9	66.5 ± 30.7	69.2 ± 47.5[a]		39.4 ± 21.6		19.6 ± 9.1
Calcium							
Filterable, mg/dL	6.5 ± 0.2	6.6 ± 0.3	6.6 ± 0.4		6.6 ± 0.5		6.4 ± 0.3
Urine, µg/min	58.0 ± 35.0	83.0 ± 41.0	150.0 ± 84.0[a]	179.0 ± 84.0[a]	162.0 ± 85.0[a]	137.0 ± 92.0[b]	101.0 ± 71.0
Reabsorption, %	99.1 ± 0.5	98.5 ± 0.7	97.9 ± 0.7[a]	97.3 ± 0.9[a]	97.5 ± 1.1[a]	97.8 ± 1.1[a]	98.5 ± 1.0
Phosphorus							
Serum, mg/dL	4.1 ± 0.4	3.6 ± 0.3	3.4 ± 0.3[b]		3.3 ± 0.5[a]		3.7 ± 0.5
Urine, µg/min	305.0 ± 281.0	305.0 ± 203.0	395.0 ± 324.0	258.0 ± 156.0	174.0 ± 106.0	118.0 ± 99.0	131.0 ± 97.0
Reabsorption, %	91.4 ± 6.2	90.3 ± 5.5	89.8 ± 6.7	94.2 ± 3.4	93.8 ± 5.4	96.4 ± 3.5	96.7 ± 2.9
Zinc							
Serum, µg/dL	87.0 ± 8.0	86.0 ± 11.0	88.0 ± 13.0		82.0 ± 12.0		82.0 ± 10.0
Urine, ng/min	300.0 ± 232.0	386.0 ± 255.0	738.0 ± 474.0[c]	739.0 ± 343.0[c]	653.0 ± 399.0	607.0 ± 321.0	863.0 ± 447.0[a]
Sodium							
Serum, mg/dL	349.0 ± 41.0	339.0 ± 35.0	337.0 ± 37.0		341.0 ± 43.0		355.0 ± 34.0
Urine, µg/min	873.0 ± 699.0	1134.0 ± 1128.0	995.0 ± 901.0	1115.0 ± 769.0	1264.0 ± 822.0	1396.0 ± 927.0	1848.0 ± 1646.0[b]
Reabsorption, %	99.7 ± 0.2	99.6 ± 0.4	99.8 ± 0.3	99.7 ± 0.3	99.5 ± 0.3	99.6 ± 0.2	99.5 ± 0.4
Potassium							
Serum, mg/dL	19.8 ± 5.3	20.0 ± 4.8	17.7 ± 5.8		18.8 ± 4.1		20.5 ± 4.5
Urine, µg/min	1226.0 ± 726.0	1713.0 ± 833.0	1129.0 ± 629.0	907.0 ± 365.0	960.0 ± 407.0	1107.0 ± 520.0	1757.0 ± 967.0
Reabsorption, %	91.7 ± 2.6	90.0 ± 3.4	94.0 ± 2.2	95.2 ± 2.0	94.2 ± 2.8	94.0 ± 2.5	90.8 ± 3.9

[1] Values are means ± SD for 13 subjects.
[2] Significantly different from baseline: a = $P < 0.001$, b = $P < 0.01$, c = $P < 0.05$.

were subjected to regression analysis. For this regression peak minus baseline values of insulin were calculated for each individual. Postprandial increases in urine calcium were corrected for baseline levels. As hypothesized there was a positive correlation ($r = 0.82$, $P < 0.01$) between the increase in urinary calcium excretion and serum insulin (**Fig. 1**). The regression was performed on 12 subjects because the data from one individual were rejected as a statistical outlier (20). Some subjects showed small or no increase in both parameters whereas others experienced large increases in both urine calcium and serum insulin after the sucrose load.

As anticipated, urinary excretion and percent reabsorption were significantly negatively correlated for the following minerals ($P < 0.001$): calcium, $r = -0.81$; phosphorus, $r = -0.85$; sodium, $r = -0.93$; potassium, $r = -0.79$. Urine calcium and serum phosphorus were weakly negatively correlated ($r = -0.41$, $P < 0.05$). The postprandial increases in urine calcium and urine zinc excretion were positively correlated ($r = 0.80$, $P < 0.01$), whereas the association between urine potassium and serum insulin was negative ($r = -0.80$, $P < 0.001$). Serum insulin was not significantly correlated with urine phosphorus, sodium or zinc.

DISCUSSION

Sucrose consumption resulted in rapid changes in the metabolism of insulin, calcium, phosphorus, sodium, potassium and zinc. Urinary excretion and percent

reabsorption were significantly negatively correlated for all minerals studied.

Ingestion of an oral sucrose load caused a marked increase in urine calcium excretion. The effect is similar to that produced by other carbohydrates (5–7) and protein (1, 2). As in the case of protein consumption (1), the increase in urinary calcium excretion after su-
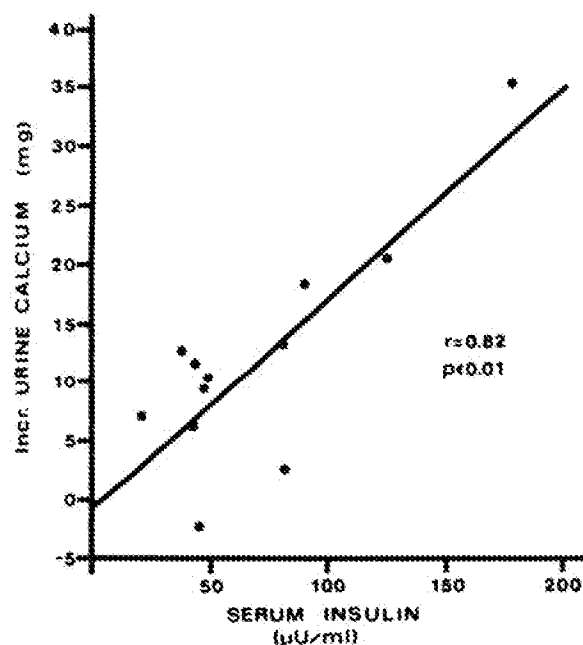

FIGURE 1 Correlation of postsucrose increases in serum insulin and urine calcium for individual subjects.

crose ingestion was associated with reduced renal calcium reabsorption and was highly correlated with the insulin response. The peak in serum insulin precedes the peak in urine calcium by approximately 0.5 h. The correlation between urinary calcium excretion and serum insulin after both protein and sucrose intake suggests that a similar mechanism is operating in both cases. We have preliminary evidence that insulin affects calcium transport in renal tubular cells [21]. In previous experiments we found that the magnitude of the calciuretic effect of protein varied greatly among individuals [1, 2]. This interindividual variability was also observed here after sucrose intake. Some subjects showed considerable insulin and urinary calcium responses to sucrose ingestion whereas others showed small increases in both parameters. The magnitude of the calciuretic response may be an important risk factor for bone loss.

We demonstrated previously that parathyroid hormone is not involved in protein-induced calciuria [22]. This conclusion is supported in the present experiment by the observation that serum phosphorus and urine calcium were weakly negatively correlated. If an inhibition of parathyroid hormone action were responsible for the sucrose-induced calciuria, serum phosphorus should have been increased, not decreased as was observed.

Glucose [23, 24] and glucose polymer [25] have been demonstrated to enhance the intestinal absorption of calcium and zinc in human subjects, but in the present research the test beverage contained no minerals except for the trace amounts present in the distilled water and sucrose. Increased absorption is therefore unlikely to explain the increased excretion of urinary calcium. In addition, intravenous infusions of glucose stimulate urinary calcium excretion in rats [3], an observation that supports a lack of intestinal involvement. Postprandial changes in blood glucose are also unlikely to have caused the calciuria because glucose infusion is calciuretic in human subjects maintained under euglycemic conditions [9] and noncalciuretic in rats if insulin secretion is blocked [3].

The time response patterns of urine sodium and potassium excretion were similar to those observed after the intake of a high protein meal [2]. Urine sodium was elevated only at $t = 3.0$ h, when the rate of urine calcium excretion was falling. An increase in the rate of urinary sodium excretion does not occur during glucose or insulin infusions shorter than 2 h [9, 10]. However, the significant correlation between urine calcium and sodium excretion observed here has also been reported in urine samples from fasted subjects consuming self-selected diets [26] and in 24-h urine samples of individuals consuming 400 mg calcium/d for 1 wk [27]. The negative correlation between serum insulin and urine potassium in the present study is consistent with the effect of insulin infusion on urine potassium reported by others [9].

Increases in urinary zinc excretion after sucrose consumption were similar to those resulting from a high protein meal [1]. There was no association between serum insulin and urine zinc, although increases in urine zinc and urine calcium were positively correlated. In dogs, insulin infusion has been shown to inhibit, and glucagon to stimulate, urinary zinc excretion [17]; measurement of both of these hormones should be made in future research on the effects of carbohydrates on the renal handling of zinc. The greater excretion of urinary zinc in the present research may have been caused by alterations in the affinity of serum zinc for its binding constituents, resulting in changes in the ultrafilterable fraction [28]. Alternatively, renal secretion of zinc across tubular cells may have been stimulated. The marked increase in urinary zinc excretion warrants further investigation.

This study has important implications. Patients with renal hypercalciuria respond to glucose ingestion by further increasing calcium excretion [29], so that in this condition the excessive consumption of sucrose might contribute to renal stone formation. Individuals who consistently respond to sucrose ingestion with a substantial increase in urinary calcium may be at greater risk of developing bone loss associated with aging. Because a postprandial calciuretic response can be induced by sucrose as well as protein, studies comparing the relative calciuretic effects of meals high in either protein, simple carbohydrate or complex carbohydrate are needed before specific dietary regimens can be recommended as beneficial to bone health.

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Plasma insulin response to oral carbohydrate in patients with glucose and lactose malabsorption

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Plasma insulin response to oral carbohydrate in patients with glucose and lactose malabsorption

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SUMMARY   Plasma insulin levels were determined following oral glucose in 12 patients
with adult coeliac disease, after oral lactose in four patients with alactasia, and in age-matched
control subjects.

In coeliac patients the insulin response was greater than expected from the small rise in blood
sugar, and no correlation was found between plasma insulin and sugar levels at any period
during the test. The separation of the plasma insulin curve from the blood sugar curve after
glucose is in keeping with the concept that a factor responsible for stimulating insulin secretion
is released from the gut during or after absorption of glucose.

In patients with selective lactose malabsorption (alactasia) administration of lactose by
mouth failed to elicit any insulin response, indicating that the insulin-releasing effect of the
bowel is not activated merely by the presence of intraluminal carbohydrate.

The availability of a sensitive immunoassay has
led to the recognition that the secretion of insulin
in vivo is closely dependent on the blood sugar
level (Yalow and Berson, 1960) and studies with
isolated pancreas preparations have shown a
similar increase in insulin output when the glucose
concentration in the incubating medium is raised
(Malaisse, Malaisse-Lagae, and Wright, 1967).

Increase in blood glucose above a threshold
level was considered to be the only important
physiological stimulus for insulin release (Field,
1964) until the demonstration by McIntyre, Holds-
worth, and Turner (1964) that the infusion of
glucose into the jejunum produced a greater rise
in insulin than an equivalent amount infused
intravenously indicated that factors other than
the blood glucose level were concerned in the
insulin response following oral glucose.

Absorption of glucose may release a humoral
substance from the bowel which then acts, to-
gether with the increase in blood glucose, in

stimulating release of insulin from islet cells
(McIntyre *et al*, 1964). An insulinotropic effect
has been attributed to various gastrointestinal
hormones (see the reviews by Holdsworth, 1969,
and Mayhew, Wright, and Ashmore, 1969) but the
mechanisms involved in gut-mediated insulin
release are not clearly understood. We have
approached this problem by studying the plasma
insulin response to oral glucose in patients with
adult coeliac disease (with subtotal villous atrophy
and malabsorption of glucose) and to the disac-
charide lactose in patients with selective lactose
malabsorption (alactasia).

Patients and Methods

COELIAC DISEASE
Twelve patients (five male and seven female),
with a mean age of 42 years, who weighed on
average 10% less than ideal for height and sex,
were studied. Four patients had started a gluten-

free diet, while the remaining eight were studied
before gluten withdrawal. Jejunal biopsy at the
time of study showed subtotal atrophy in all
patients.

Standard 50g oral glucose tolerance tests were
performed after an overnight fast. Venous blood
was taken for blood sugar and insulin estimations
were made at 0, 15, 30, 60, 90, and 120 minutes.
The results were compared with those from 12
age-matched controls who were all within 10% of
ideal body weight.

In seven coeliac patients and seven control
patients an intravenous glucose tolerance test
was also performed. Venous blood was removed
while the patients were fasting and at 10, 20, 30,
40, 50, and 60 minutes after 25g intravenous
glucose.

Fig. 1 *Sugar and insulin response to intravenous glucose (25g) in seven control subjects (left) and seven coeliac patients (right). (Here and in Figures 2 and 4, ●————● denotes plasma insulin and ×----× blood sugar.)*

ALACTASIA

Four patients (all male) with a mean age of 35
years were studied. The diagnosis of alactasia
had been made on a history of milk intolerance,
confirmed by lactose tolerance tests and jejunal
lactase activity of under 0·3 units/g mucosa (lower
limit of normal lactase activity for our laboratory
1·0 unit/g mucosa) (Ferguson and Maxwell, 1967).
In each case small-bowel histology was normal. In
these patients venous blood was taken at 0, 15,
30, 60, 90, and 120 minutes after 100g oral lactose
and results were compared with four age-matched
normal control subjects. None of the patients
nor control subjects studied were diabetic.

Blood sugar was measured as total reducing
substances on a Technicon AutoAnalyzer. Plasma
insulin was assayed by the immunoprecipitation
technique of Hales and Randle (1963), using
standards and antisera previously described
(Buchanan and McKiddie, 1967a).

Fig. 2 *Sugar and insulin response to oral glucose (50g) in 12 control subjects (left) and 12 coeliac patients (right).*

Results

INSULIN RESPONSE TO GLUCOSE

The effect of intravenous glucose is shown in
Fig. 1, and demonstrates a virtually identical
sugar and plasma insulin response in control and
coeliac groups. There is thus no evidence for a
delayed or impaired pancreatic response to the
stimulus of hyperglycaemia in the coeliacs.

In control subjects the sugar and insulin curves
following oral glucose closely parallel each other
(Fig. 2). In the coeliac patients the sugar curve
is much flatter (mean maximal rise of only 11
mg%) consistent with glucose malabsorption,
the insulin rise is slower than in the controls,
with a delayed peak, and the curve does not
parallel the sugar curve. Coeliac patients were
found to have a significantly lower sugar level at
15 minutes and 30 minutes after oral glucose
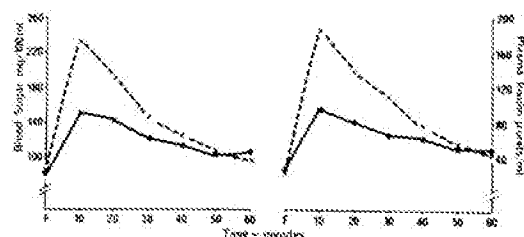than controls (p < 0·01), but a statistically signi-

Fig. 3 *Sugar and insulin areas after oral glucose in control subjects (black area) and coeliac patients (dotted area).*
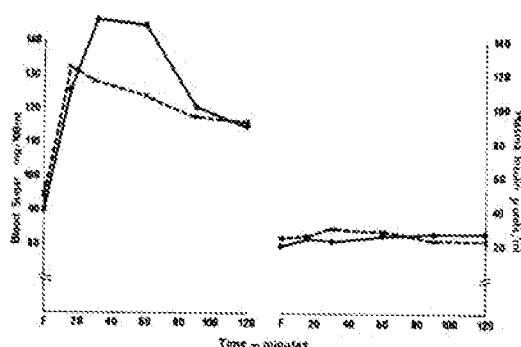
Fig. 4   *Sugar and insulin response to oral lactose (100g) in control subjects (left) and patients with alactasia (right).*

ficant difference in insulin levels occurred only at 15 minutes following oral glucose ($p < 0.05$).

The areas below the sugar and plasma insulin response curves (calculated as mg/min/100 ml for sugar and microunits/min/ml for insulin) give some measure of sugar absorption and plasma insulin secretion (Kalkhoff, Schalch, Walker, Beck, Kipnis, and Daughaday, 1964; Perley and Kipnis, 1965). The sugar and insulin areas expressed as percentages of control values are shown in Figure 3. It can be seen that although the overall insulin response in coeliacs is decreased to 65% of the control value, this is out of proportion to the sugar response which is only 22% of the response in control patients.

A significant positive correlation was found between sugar and insulin values in control subjects at 60 minutes ($r = 0.67$), 90 minutes ($r = 0.70$), and 120 minutes ($r = 0.83$) after oral glucose. In the coeliac patients on the other hand, where little rise in blood sugar occurs, no correlation was found between sugar and insulin levels at any time during the test period.

INSULIN RESPONSE TO LACTOSE
Figure 4 shows the effect of oral lactose on the sugar and insulin response in patients with alactasia, and in the control group. In the alactasia group the sugar curve is flat (lactose malabsorption) and there was no insulin response, while in controls there is a normal rise in sugar and insulin levels after oral lactose.

Discussion

A positive correlation between sugar and insulin levels has been found in the latter part of the oral glucose tolerance test in normal control subjects in this and other studies (Buchanan and McKiddie, 1967b; Martin, Pearson, and Stocks, 1968). These findings are consistent with the view that

insulin secretion at this stage is determined by a direct effect of the elevated blood sugar on islet cells, as has been shown with isolated pancreas preparations (Malaisse *et al*, 1967). On the other hand the absence of correlation between these variables in the first hour after glucose ingestion suggests that insulin secretion during the initial period of the glucose test may be largely the result of a factor or factors other than the blood sugar level, and an insulin release mechanism mediated by the gut may provide the explanation for this early insulin response.

In coeliac patients where glucose absorption is greatly diminished (Holdsworth and Dawson, 1965) and the increment in blood sugar above fasting levels is small, no correlation is found at any stage in the test between sugar and insulin values. It is interesting to speculate whether the insulinotropic effect of the bowel might be the major factor in determining insulin secretion throughout the test period in these patients with glucose malabsorption.

These studies provide further evidence for the existence of an insulin-releasing effect by the alimentary tract. The site of release of the insulin-stimulating factor remains unknown, but it is unlikely to originate from the liver or portal circulation (McIntyre, Turner, and Holdsworth, 1968; Holdsworth, 1969).

We have shown that the stimulus to the release of an insulinotropic factor does not depend merely on mucosal contact or indirect changes (such as in *p*H, motility, or osmotic pressure) evoked by the passage of carbohydrate into the lumen of the small bowel, for the presence of unabsorbed lactose in the gut of lactase-deficient patients did not produce a rise in plasma insulin. This suggests that it may be the onset of monosaccharide absorption which stimulates the bowel-mediated release of insulin after oral carbohydrate. As there is experimental evidence that insulin can enhance the absorption of glucose from the small intestine *in vitro* (Love and Canavan, 1968) such an interrelationship could provide a feedback system for facilitating glucose absorption.

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Effect of Protein Ingestion on the Glucose and Insulin Response to a Standardized Oral Glucose Load

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Type II diabetic subjects were given 50 g protein, 50 g glucose, or 50 g glucose with 50 g protein as a single meal in random sequence. The plasma glucose and insulin response was determined over the subsequent 5 h. The plasma glucose area above the baseline following a glucose meal was reduced 34% when protein was given with the glucose. When protein was given alone, the glucose concentration remained stable for 2 h and then declined. The insulin area following glucose was only modestly greater than with a protein meal (97 ± 35, 83 ± 19 μU · h/ml, respectively). When glucose was given with protein, the mean insulin area was considerably greater than when glucose or protein was given alone (247 ± 33 μU · h/ml). When various amounts of protein were given with 50 g glucose, the insulin area response was essentially first order. Subsequently, subjects were given 50 g glucose or 50 g glucose with 50 g protein as two meals 4 h apart in random sequence. The insulin areas were not significantly different for each meal but were higher when protein + glucose was given. After the second glucose meal the plasma glucose area was 33% less than after the first meal. Following the second glucose + protein meal the plasma glucose area was markedly reduced, being only 7% as large as after the first meal. These data indicate that protein given with glucose will increase insulin secretion and reduce the plasma glucose rise in at least some type II diabetic persons. DIABETES CARE 1984; 7:465–70.

It is well known that protein ingestion or the administration of amino acids orally or intravenously will stimulate insulin secretion in normal or mildly diabetic subjects.[1-5] In normal subjects we have previously demonstrated that a diet composed of 40% of the food energy in the form of protein and 20% in the form of carbohydrate results in a clear increase in circulating insulin concentration after each meal. This occurred in the absence of a significant rise in glucose concentration after the second and third meals of the day.[6] This study suggested that a moderately high protein diet might be useful in the treatment of type II diabetic patients. To test this hypothesis, we have determined the plasma glucose and insulin response to a standard glucose meal in the absence and the presence of varying amounts of protein in mild type II diabetic subjects.

SUBJECTS AND METHODS

Nine male, untreated diabetic subjects were studied in a metabolic unit. All patients met the National Diabetes Data Group criteria[7] for the diagnosis of type II diabetes. The mean age was 61 ± 12 yr with a range of 38–74 yr. The mean percent of desirable body weight was 123 ± 23% using the 1959 Metropolitan Life Insurance Co. tables for persons of medium frame. All subjects signed an informed consent and the study was approved by the hospital committee on human subjects. All participants were on diets consisting of at least 200 g of carbohydrate/day with adequate food energy for 3 days before testing. None of the subjects had received treatment with either oral hypoglycemic agents or insulin previously. After an overnight fast of 8–10 h, an indwelling catheter was inserted into an antecubital vein and kept patent with small amounts of heparin.

The plasma glucose was determined by a glucose-oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, California). Serum immunoreactive insulin (IRI) was measured in duplicate by a standard radioimmunoassay method[8] using a kit supplied by Pharmacia Laboratories (Piscataway, New Jersey). The glucose and insulin areas above the fasting baseline were determined by planimetry. Areas below the baseline were subtracted from areas above the baseline to give a net area. The fat content of the ham-
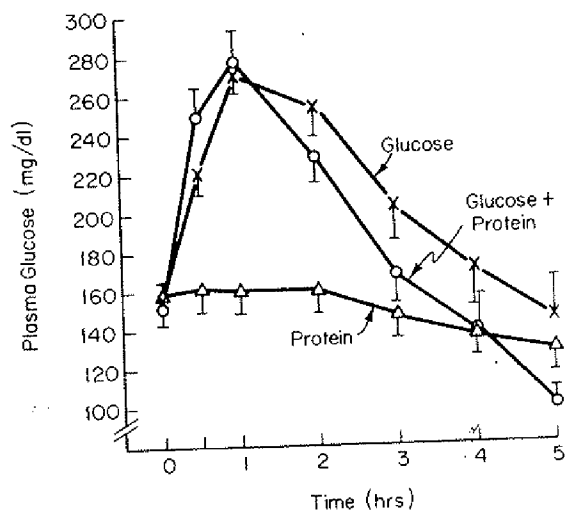
FIG. 1. *Plasma glucose response to ingestion of 50 g glucose, 50 g protein, or a combination of 50 g glucose and 50 g protein. Seven male, untreated diabetic subjects were studied.*

burger was determined by gravimetric analysis of several ether extractions of the meat. Student's t-test for paired variates was used for analysis of statistical significance. Data are presented as mean ± standard error of the mean.

The following three studies were done:

I. Seven type II diabetic subjects were given 50 g of glucose (Glutol, Paddock Laboratories, Minneapolis, Minnesota) or 50 g of protein or a combination of 50 g of glucose with 50 g of protein over 3 consecutive days in a random order. In all studies, protein was given as well-cooked very lean hamburger (236 g raw wt). Blood for glucose and insulin meas-

FIG. 2. *Serum insulin response to ingestion of 50 g glucose, 50 g protein, or a combination of 50 g glucose and 50 g protein. The subjects are the same as indicated in Figure 1.*

urements was drawn at 0, ½, 1, 2, 3, 4, and 5 h after the ingestion of the test meal.

II. In five type II diabetic subjects the effect of adding 10, 30, and 50 g protein to a standard 50-g glucose dose was studied. Protein was given as well-cooked very lean (6.5% fat) hamburger (47, 142, and 236 g raw wt, respectively). Blood samples for glucose and insulin were collected at the time intervals indicated above.

III. Five type II diabetic subjects were given either two doses of 50 g of glucose 4 h apart or 50 g glucose with 50 g protein 4 h apart. Blood for glucose and insulin measurements was drawn before and ½, 1, 2, 3, 4, 5, 6, 7, and 8 h after the ingestion of the first test meal.

RESULTS

Following ingestion of 50 g glucose, the plasma glucose rapidly increased from the baseline of 156 ± 13 mg/dl to a peak of 271 ± 9.8 mg/dl at 1 h. It had returned to the baseline by about 4–5 h (Figure 1). When 50 g protein alone was given, there was no change in plasma glucose concentration for 2 h and then it began to decline gradually. By 5 h it was approaching a normal fasting level. When glucose and protein were given together, the plasma glucose concentration reached the same peak concentration as with glucose alone. However, the glucose concentration declined more rapidly. By 3.5 h it had returned to the baseline. It continued to
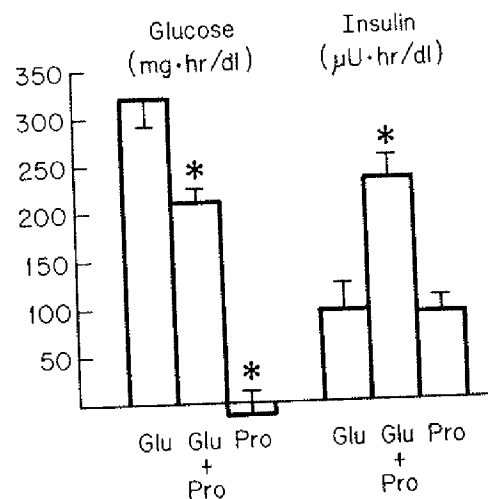
FIG. 3. *Areas above baseline for plasma glucose and serum insulin determined over the 5-h period following ingestion of glucose (glu), protein (pro), or a combination of glucose and protein. [*Indicates statistical difference from glucose administration alone for glucose area. For insulin area glucose + protein is significantly greater than glucose or protein ingestion individually. The glucose and protein area is also significantly greater than the sum of glucose alone plus protein alone (P < 0.01). This indicates synergism between oral glucose + oral protein ingestion in the stimulation of insulin secretion.] The subjects the same as indicated in Figure 1.*
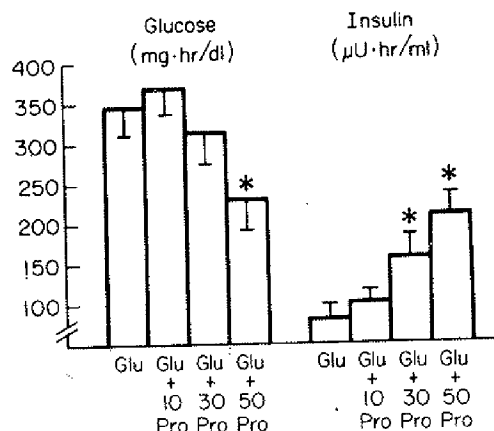
FIG. 4. Areas above baseline for plasma glucose and serum insulin determined for 5 h following ingestion of 50 g glucose and increasing amounts of protein. (*Indicates results statistically different from those obtained after glucose ingestion alone.) Five male, untreated diabetic, subjects were studied.

FIG. 5. Plot of insulin areas obtained with increasing amounts of protein ingested. The subjects are the same as indicated in Figures 1 and 4.

decline such that by 5 h it was well within the normal fasting glucose range (99.5 ± 7 mg/dl) (Figure 1).

Following glucose ingestion the mean serum insulin concentration rose to a maximum of 53 ± 13 μU/ml at 1 h and then gradually decreased but had not reached the baseline concentration by 5 h (Figure 2). When only protein was given, the peak in insulin concentration occurred later than when glucose was given (2 h and 1 h, respectively). The peak also was not quite as high (45 ± 6.9 μU/ml) as when glucose was given, although the difference did not reach statistical significance (Figure 2). By 5 h it also had not declined to the fasting level. When glucose was given with protein, the insulin peak (96 ± 18 μU/ml) was significantly greater than when glucose or protein was given individually. The peak occurred at 2 h, i.e., the same as when protein was given alone. By 5 h the insulin concentration was still considerably elevated. In fact, it was more than twice as high (38 ± 10 μU/ml) as the fasting value (16 ± 2 μU/ml).

The areas of the glucose and insulin curves above the baseline were determined and are shown in Figure 3. The glucose area after glucose plus protein ingestion was only 65% of the area observed with glucose alone (Figure 3) and the difference was statistically significant (P < 0.05). The glucose area following protein ingestion was slightly negative as expected. The insulin areas following glucose alone or protein alone were quite similar (93 ± 33 μU · h/ml and 87 ± 18 μU · h/ml, respectively). However, the area after the ingestion of glucose combined with protein was much greater (233 ± 39 μU · h/ml). In fact, it was approximately 2.5 times as high as with either glucose or protein alone. It was 30% greater than the sum of the areas of glucose and protein added together.

Addition of 10 g protein to a 50-g glucose load did not significantly affect the glucose curve (Figure 4). With addition of 30 g protein, the mean was modestly, but not
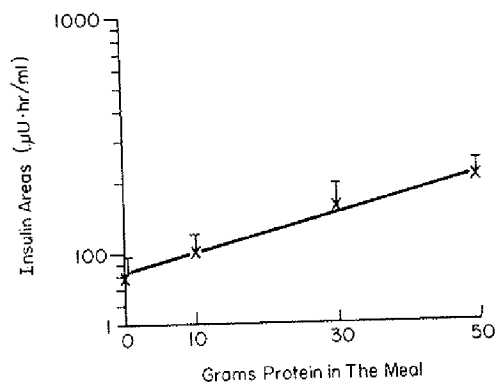
significantly, reduced. With addition of 50 g protein, there was a further reduction that was statistically significant (P < 0.05).

The mean serum insulin area following 10 g protein with 50 g glucose was 125% of that obtained with glucose alone (Figure 4). However, this did not reach statistical significance. There was a further increase in insulin area when 30 and 50 g of protein were given with glucose, and these increases were statistically significant. When the dose response was plotted on semilog paper (Figure 5), the curve approximated a straight line, suggesting a first-order relationship between the response and dose of protein given. The calculated K (slope) was 2.8 μU · h/g protein/ml. However, there was a suggestion of a falloff in response at the 50-g dose.

The plasma glucose and insulin response to two sequential doses of glucose given 4 h apart is shown in Figure 6. The rise in plasma glucose concentration was modestly smaller after the second dose, although the difference was not statistically significant. The insulin curve after the second meal also was similar to the first. However, the rise started at a
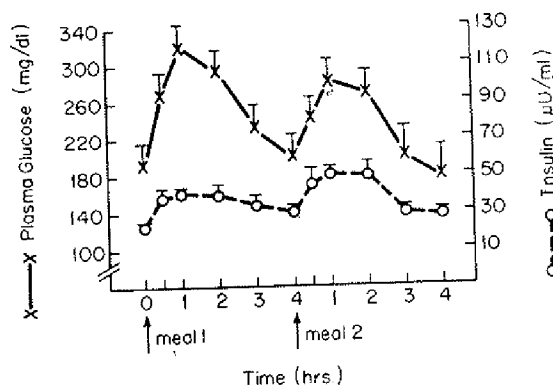
FIG. 6. Plasma glucose and serum insulin responses to 50 g glucose ingested twice, 4 h apart. Five male, untreated diabetic subjects were studied.

FIG. 7. Areas above baseline for plasma glucose and serum insulin determined over the 4-h period following consecutive 50-g glucose meals given 4 h apart (see Figure 6). The subjects are the same as indicated in Figure 6.

higher level than after the first meal. When the areas above the fasting baseline were determined, the glucose area after the second meal was significantly reduced compared with the first meal (307 ± 29 versus 202 ± 32 mg · h/dl). The insulin areas after each meal were not significantly different (58 ± 11 versus 82 ± 10 μU · h/ml, respectively) (Figure 7).

When glucose was given with protein 4 h apart (Figure 8), the peak glucose concentration after the second meal was considerably less than after the first meal. As expected, the rise in insulin concentration was greater than with the glucose administration alone. The rise after the second meal was less than after the first meal, but peak concentration after each meal was similar. The first glucose area (Figures 8 and 9) was less than when glucose was given alone (Figures 6 and 7) and the area was further reduced after the second meal compared with the first (17 ± 6 versus 230 ± 38 mg · h/dl). Actually, the second area was only 7% of the first. The insulin areas after the first (207 ± 26 μU · h/ml) and second (197 ± 37 μU · h/ml) meals were essentially identical (Figure 9). Compared with the response to glucose alone, the insulin area was 3.6-fold greater after the first meal and 2.4-fold greater after the second meal.

DISCUSSION

That protein stimulates insulin secretion in mild type II diabetic subjects is in agreement with previous studies.[4,5,9] In the present study, the stimulation of insulin secretion by protein in the form of hamburger was similar on a weight basis to that of glucose, although the peak response was delayed. In addition, the insulin response to co-ingestion of glucose and protein was

greater than the sum of the responses to glucose and protein added together. This indicates a strong synergism between oral protein and glucose in the stimulation of insulin secretion. When increasing amounts of protein were given with a standard amount of glucose, the insulin response was first order in regard to the quantity of protein ingested. Thus, smaller amounts of protein were relatively more potent than larger amounts. A synergistic effect on insulin secretion also has been reported previously in four normal women who received beef steak with glucose. However, in this study there was little change in insulin concentration when steak containing approximately 60 g of protein was given alone.[10]

The hamburger given contained 6.5% fat. Dietary fat has been reported to delay gastric emptying.[11] Thus, the fat present could have affected the results obtained. A major effect on gastric emptying is not likely, however, since the peak glucose concentration following ingestion of the hamburger with glucose was similar to that observed with glucose ingestion alone (Figure 1). Also, the peak occurred at similar times, and the decrease in glucose concentration was more rapid when the combination was ingested. We would not have expected the increased food-energy load[12] following the ingestion of hamburger with glucose to have affected gastric emptying of glucose for the same reasons. Nevertheless, since the rate of gastric emptying was not measured, a small effect on the glucose response cannot be completely ruled out.

In a large group of normal subjects Floyd et al.[2] reported a rise in insulin concentration similar to that observed in the present study. The total amount of protein given was not stated, but calculations based on the usual protein concentration in lean beef and chicken livers, the foods used in the study, indicate that it was at least twice as much as was used in the present study. In a subsequent study[4] the same group reported the response to a protein meal in subjects with glucose intolerance and type II diabetes compared with normal subjects. In general, the insulin response to the protein meal correlated with the insulin response to a standard glucose meal. In mildly obese, glucose-intolerant subjects
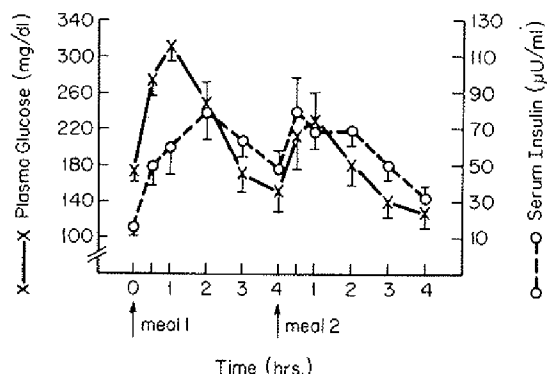
FIG. 8. Plasma glucose and serum insulin responses to 50 g glucose + 50 g protein ingested twice, 4 h apart. The subjects are the same as indicated in Figure 6.
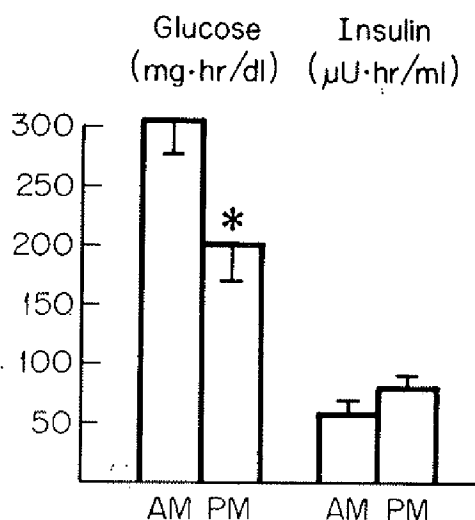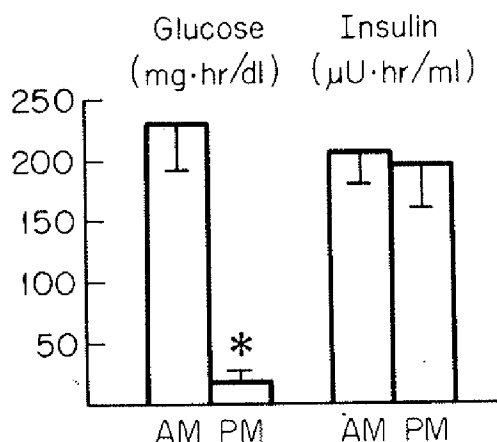
FIG. 9. *Areas above baseline for plasma glucose and serum insulin determined over the 4-h period following consecutive ingestion of 50 g glucose with 50 g protein given 4 h apart. (see Figure 8). (* Indicates response to second meal statistically significantly different from response to first meal.) The subjects are the same as indicated in Figure 6.*

insulin response to glucose was greater than in normal subjects. The response to a protein meal also was greater. In the normal-weight diabetic subjects the insulin response to the standard glucose meal was less than in the normal subjects. The response to the protein meal also was less. They suggested that the exaggerated insulin response to a protein meal in diabetic subjects compared with normal subjects reported by Berger and Vongaraya[5] was due to the use of obese, diabetic patients in their study, since the insulin response to a standard glucose meal also was greater in the diabetic subjects. However, review of the data indicates that the insulin response to glucose was only modestly greater, whereas the response to protein was considerably greater than in the normal subjects.

Thus, whether the insulin secretory response to protein ingestion in type II subjects is relatively better maintained than the secretory response to glucose ingestion is controversial. Our data would suggest that the response to protein is better maintained in at least some type II diabetic subjects. This is supported by data obtained in partially pancreatectomized rats.[13]

That a high protein content might be useful in the diet for diabetic persons was suggested as early as 1936.[14] It was based on the observation that ingestion of up to 140 g of protein as lean beef did not significantly increase the blood glucose concentration in either normal or mild type II diabetic subjects even though theoretically a large proportion of the amino acids was available for conversion to glucose.[14] Our data also indicate that protein ingestion does not cause an increase in plasma glucose and in fact may reduce the glucose concentration (Figure 1). In addition, when protein was given with glucose, the postmeal glucose area was reduced. Similar results have been reported in both normal[10] and type II diabetic subjects.[9] However, this has not been a universal ob-

servation. Jenkins et al.[15] have reported that addition of protein to a carbohydrate meal does not reduce the plasma glucose area above the baseline in normal subjects. Also, Day et al.[16] reported that addition of varying amounts of protein to a constant amount of carbohydrate in a meal did not significantly influence the plasma glucose rise. When there was less than 8 g of protein in the meal, the insulin response per unit increase in plasma glucose concentration was less than with a larger amount of protein; otherwise, they also noted little difference in insulin response as the protein content was increased up to as much as 25 g. When a greater amount of protein was given, both the glucose and insulin responses were increased. In the latter study the subjects were only studied for 90 min and the meal was given at noon.

It has been known for many years that giving normal individuals a second glucose meal approximately 4 h after a previous glucose meal results in an improved rate of glucose clearance.[17-19] This is the so-called Staub–Traugott effect. We were certainly interested in determining if this effect would be observed when protein was given with glucose to diabetic subjects for three reasons. First, we had demonstrated a much greater insulin rise when protein was given with glucose as a single meal than when glucose was given alone. Second, we had observed a greater plasma glucose area above baseline and smaller insulin area above baseline when mild type II diabetic subjects were given 50 g of glucose compared with a mixed breakfast meal containing approximately 70 g of carbohydrate and approximately 20 g of protein.[20] Lastly, in normal subjects receiving a 40% protein diet, we have previously demonstrated a reduced glucose response after a second and third meal were given 4 h apart.[6] In these subjects following each meal there was a distinct, sharp rise in insulin that could not be accounted for by a rise in glucose concentration or amino acids.[21]

In the present study, when glucose was given alone, a second meal effect (Staub–Traugott effect) was clearly observed even though the plasma insulin areas were similar after each meal (Figure 6). When protein was given with glucose, the second meal effect was much more striking. The second meal area was only 7% of that after the first meal. The insulin areas after each meal were essentially identical, although they were considerably higher than with glucose administration alone.

It is well known that protein ingestion stimulates a rise in circulating glucagon concentration; with glucose ingestion it is depressed.[22-26] It is also clear that the circulating glucagon concentration depends on the ratio of protein to carbohydrate in the meal. If the protein-to-carbohydrate ratio is high, it will increase, whereas if the ratio is low, it will decrease.[16,21] We had planned to determine the glucagon response in our studies; unfortunately, the tubes containing plasma samples for glucagon determination were either broken and/or samples thawed when our laboratory was moved to another building. Thus, it was not possible to determine the glucagon response in these subjects. From previous experience, we would anticipate only a modest rise in glucagon when equal

amounts of protein and glucose are given.[21] In any regard, the insulin secreted in response to the mixture of protein and glucose was sufficient to reduce the postmeal glucose rise.

Whether a moderately high protein will be beneficial in type II diabetic patients remains to be determined. Nevertheless, the present studies suggest that protein ingestion is important in stimulating insulin secretion in these individuals. When carbohydrate is ingested, the simultaneous ingestion of protein may also prove useful in reducing the postmeal glucose rise. In addition, these data indicate the need to consider the insulin secretory response and a second and third meal effect on blood glucose concentration when determining the glycemic response to a food.

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The Metabolic Response to Ingestion of Proline With and Without Glucose

Frank Q. Nuttall, Mary C. Gannon, and Kelly Jordan

Ingested protein results in an increase in circulating insulin and glucagon concentrations and no change, or a slight decrease, in circulating glucose. In subjects with type 2 diabetes, when protein is ingested with glucose, insulin is further increased and the glucose rise is less than when glucose is ingested alone. Presumably these effects are due to the amino acids present in the proteins. The effects of individual amino acids, ingested in physiologic amounts, with or without glucose, have not been determined. Therefore, we have begun a systematic study of the response to ingested amino acids. Eight young, non-obese, subjects (4 men, 4 women) ingested 1 mmol proline/kg lean body mass, 25 g glucose, 25 g glucose + 1 mmol proline/kg lean body mass or water only on 4 separate occasions at 8 AM. Blood was obtained before and after ingestion of the test meal over the following 150 minutes. Proline ingestion resulted in a 13-fold increase in the plasma proline concentration. This was decreased by 50% when glucose was ingested with proline. Proline alone had little effect on glucose, insulin, or glucagon concentrations. However, ingestion of proline with glucose resulted in a 23% attenuation of the glucose area response and no change in insulin response compared with the response to that of glucose alone. A glucose-stimulated decrease in glucagon was further facilitated by proline. Ingested proline is readily absorbed. It reduces the glucose-induced increase in glucose concentration in the presence of an unchanged insulin and a decreased glucagon response.
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DIETARY PROTEIN ingestion clearly results in an increase in circulating insulin and glucagon in people with and without type 2 diabetes.[1] However, it does not result in an increase in glucose concentration even though the amino acids resulting from digestion of the protein can be converted into glucose through gluconeogenesis.[2] Indeed, protein ingested with glucose may actually diminish the glucose response to the ingested glucose.[3,4] Nevertheless, the insulin and glucose response to individual protein sources varied considerably.[3]

Because proteins are composed of 20 different amino acids and the composition of each protein is different, we have begun a systematic study of the effect of ingested individual amino acids on the circulating glucose, insulin, and glucagon concentrations when ingested with and without 25 g glucose. The amount of amino acid ingested in each case is 1 mmol/kg lean body mass. The subjects are normal young adults.

We realize that the metabolic response to intact proteins may be different from that resulting from ingestion of the individual constituent amino acids. Nevertheless, we think evaluating the response to individual amino acids should be the first step in trying to understand how proteins stimulate insulin secretion and lower the blood glucose concentration after glucose ingestion and why the response varies among different protein sources.

The results from the ingestion of arginine[5] and glycine[6] have been published previously. Others have reported that alanine administered orally also increases plasma insulin,[7] with little change in plasma glucose. The effect of ingestion of alanine with glucose was not studied. The response to ingestion of proline is presented in this report.

MATERIALS AND METHODS

Four men and 4 women were studied. All subjects were nondiabetic, based on the National Diabetes Data Group criteria for the diagnosis of diabetes. The mean age of the subjects was 28 (range, 22 to 38), and mean body mass index (BMI) was 23 (range, 21 to 34). Mean body weight was 80 kg (range, 58 to 107 kg).

Written informed consent was obtained from all subjects, and the study was approved by the Department of Veterans Affairs Medical Center and the University of Minnesota Committee on Human Subjects. All subjects had fasted 12 hours overnight before testing.

Each subject was admitted to the Special Diagnostic and Treatment Unit (SDTU) on the morning of the study. Body composition was determined using bioelectrical impedance (RJL Systems, Detroit, MI).

An indwelling catheter was inserted into a forearm vein and kept patent with intravenous saline. Baseline blood samples were drawn at 7:30 AM, 7:40 AM, and 7:50 AM. At 8 AM, subjects ingested 1 of 4 test solutions in random order. The test solutions consisted of: (1) 1 mmol proline/kg lean body mass, (mean 6.0 g [53 mmol] with a range of 5.9 g [41 mmol] to 8.2 g [71 mmol]); (2) 25 g glucose (45 mL Glutol); (3) 1 mmol proline/kg lean body mass plus 25 g glucose; or (4) water only. The pH of the amino acid containing solutions was adjusted to 7.0. All subjects ingested all 4-test solutions. The volume of the solutions ingested was 150 mL and the time for ingestion was less than 1 minute. Blood was obtained every 10 minutes after ingestion of the test solution for 120 minutes and then again at 150 minutes.

There is some evidence that protein has an effect on satiety.[8] Therefore, we were interested in whether individual amino acids also could affect satiety. To assess this, the subjects were asked to complete a satiety index after the final blood draw (155 minutes after ingestion of test solution). The satiety index consisted of the following 5 questions: (1) How strong is your desire to eat? (2) How hungry do you feel? (3) How full do you feel? (4) How much food do you think you could eat? (5) How pleasant have you found the test substances? The subjects provided a numerical response using a linear scale of 1 to 100 with 1 being the least and 100 being the most. They then were served a regular mixed meal with more food energy than the subjects could eat and the amount of food energy (kcal) ingested was calculated by the study

dietician using Nutritionist V as a reference. The 4-day study was conducted over a 2-week period.

Plasma glucose was determined by a glucose oxidase method using a Beckman glucose analyzer with an O_2 electrode (Beckman Instruments, Fullerton, CA). Serum immunoreactive insulin was measured using an automated chemiluminescent assay on DPC's IMMULITE machine (Diagnostic Products, Los Angeles, CA). Glucagon was determined by radioimmunoassay (RIA) using kits purchased from Linco (St Louis, MO). Alpha amino nitrogen (AAN) was determined by the method of Goodwin.[9] Individual amino acid concentrations were determined by high-performance liquid chromatography (HPLC) in the laboratory of Dr K.S. Nair at the Mayo Medical School, Rochester, MN.

The net 150-minute area responses, ie, areas above baseline, were calculated using a computer program based on the trapezoid rule.[10] Food energy consumption was calculated using the computer software Nutritionist V (Hearst, San Bruno, CA). Statistics were determined using Student's t test for paired variates, analysis of variance (ANOVA), or Wilcoxon's signed-rank with the StatView 512+ program (Abacus Concepts, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA), as appropriate. A P value of $\le$ .05 was the criterion for significance. Data are presented as means $\pm$ SEM.

RESULTS

After the subjects ingested proline alone the proline concentration increased rapidly and reached a concentration approximately 13-fold over the initial concentration. Ingested glucose had little effect on the proline concentration. However, when glucose was ingested with proline, it reduced the proline area response by 50% (Fig 1).

The mean fasting glucose concentration was 4.6 $\pm$ 0.13 mmol/L (82 $\pm$ 2.3 mg/dL). After ingestion of glucose alone, the glucose concentration increased steadily to 7 mmol/L (126 mg/dL) at 40 minutes. It remained elevated for 30 minutes before gradually returning to the fasting concentration at approximately 150 minutes. After ingestion of glucose plus proline, the glucose concentration increased at the same rate, but reached a peak earlier (30 minutes) when compared with glucose alone. The maximum concentration also was lower (6.2 mmol/L or 111 mg/dL). After the ingestion of proline alone, the glucose concentration was essentially unchanged from that when only water was ingested (Fig 2A).

When proline was ingested with glucose, the glucose area response above baseline was attenuated by 23% when compared with the ingestion of glucose alone. This was statistically significant (P < .05). The area response to proline alone was not different from the area response to water ingestion (Fig 2B).

The mean fasting serum insulin concentration was 8.5 $\pm$ 1.7 μU/mL (51 $\pm$ 10 pmol/L). When subjects ingested glucose alone, the serum insulin concentration increased to a maximum of 33 μU/mL (199 pmol/L) at 30 minutes. It remained elevated for 30 minutes before gradually decreasing to a final concentration of 12 $\pm$ 4.6 μU/mL (72 $\pm$ 28 pmol/L) at 150 minutes. After the ingestion of glucose with proline, the serum insulin concentration reached a peak of 41 $\pm$ 13 μU/mL (246 $\pm$ 76 pmol/L). The peak occurred earlier (20 minutes) and remained elevated at this concentration for 20 minutes over that observed when only glucose was ingested. It then returned to the fasting concentration at 150 minutes. When proline was ingested alone, there was a slight, but persistent, increase in serum insulin concentration (Fig 3A).

Fig 1. (A) Mean ($\pm$ SEM) plasma proline concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated areas under the curve (AUC) using the fasting values as baseline. *Significantly different from glucose + proline using Student's t test (P < .008).

The mean 150-minute integrated serum insulin area response was 11% greater after subjects ingested glucose plus proline compared with when glucose was ingested alone. This was not significant (P = .29). When analyzed over 50 minutes as an index of the more rapid increase in insulin, the difference was 20% greater than when subjects ingested glucose alone. This was statistically significant (P = .047). In addition, the rate of increase in insulin concentration determined by slope analysis was statistically significantly greater after glucose + proline compared with proline alone (P < .04). This increased rate occurred even though the proline increase was greatly diminished (Fig 1A). The insulin area response to ingested proline alone compared with water integrated over 150 minutes, although small, was statistically significant (P < .02) (Fig 3B). In fact, it was greater in every individual.

The initial mean fasting glucagon concentration varied modestly. However, the overall mean was 62 $\pm$ 9 pg/mL. After the subjects ingested glucose, the glucagon concentration decreased as expected. A similar result was seen after ingestion of glucose plus proline. The mean glucagon concentration remained below the fasting concentration throughout the period of study. After the ingestion of proline alone, the mean gluca-
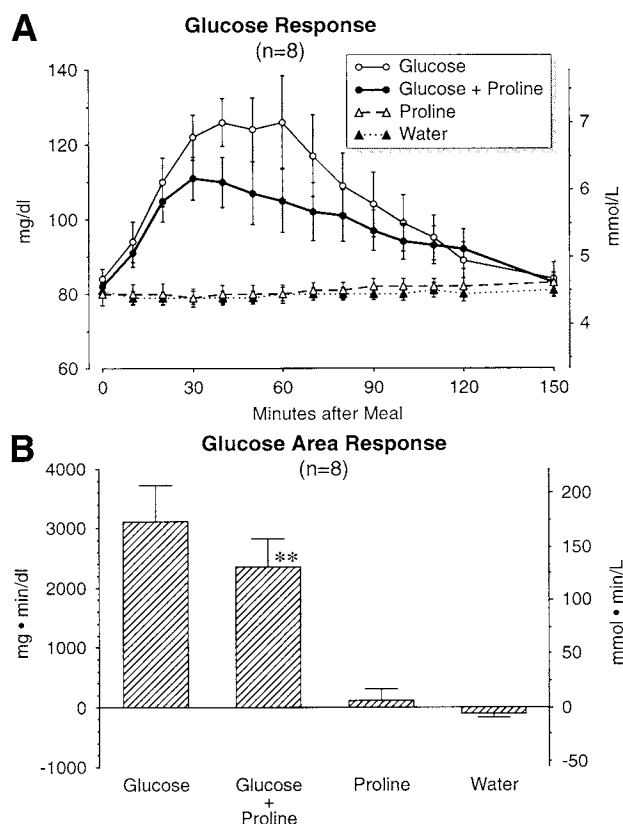
Fig 2. (A) Mean (± SEM) plasma glucose concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Student's *t* test (*P* < .05).

gon concentration gradually decreased and generally the decrease was greater than after water ingestion (Fig 4A).

The mean integrated glucagon area response was negative after glucose ingestion. The mean glucose area response after the ingestion of proline with glucose also was negative. This was more negative than with glucose alone, and the difference was statistically significant using Wilcoxon's signed-rank analysis (*P* = .03). Proline ingested independently also resulted in a decrease in glucagon area response, but this did not quite reach statistical significance using Wilcoxon's signed-rank analysis (*P* = .08) (Fig 4B).

The mean fasting AAN concentration was 3.7 ± 0.1 mg/dL. The AAN concentration decreased after the ingestion of glucose alone, whereas, it remained unchanged after water ingestion. The AAN concentration increased promptly after proline ingestion, reached a maximum at 30 minutes, and then decreased, but was still elevated at the end of the study. When glucose was ingested with the proline, the increase was attenuated by approximately 50%, but the elevation in concentration remained for the duration of the study (Fig 5A).

The mean integrated negative AAN area response after glucose ingestion was statistically significant when compared with water ingestion alone (*P* < .02). The smaller increase in AAN

area after ingestion of proline with glucose, compared with ingestion of only glucose, was highly significant (*P* < .001) (Fig 5B).

The response of individual amino acids to ingestion of water, glucose, praline, and proline with glucose is shown in Table 1. The concentrations of leucine, isoleucine, and valine decreased after ingestion of glucose alone or after ingestion of glucose with proline, but not when proline alone was ingested. The concentration of the other amino acids (serine, tyrosine, alanine, glycine, phenylalanine, methionine, threonine, cysteine, glutamine, lysine, arginine, histidine, and glutamate) did not change significantly after ingestion of proline, glucose, or proline with glucose (data not shown). Because blood was drawn from a forearm vein, rather than from an arterialized hand vein, it is possible that part of the effect noted could have been due to local extraction of glucose and/or amino acids by forearm tissues. However, we doubt that this was significant because the venous catheter generally was inserted just above the wrist.

The subjects' sensory response to the ingested substances is shown in Table 2. The subjects' desire to eat was significantly lower by rank sum analysis (*P* = .05) after subjects ingested proline alone and after glucose plus proline (*P* = .002), but not after glucose alone (rank sum *P* = 0.1) when compared with water only. The subjects also reported a greater degree of fullness after ingestion of proline alone, proline plus glucose,
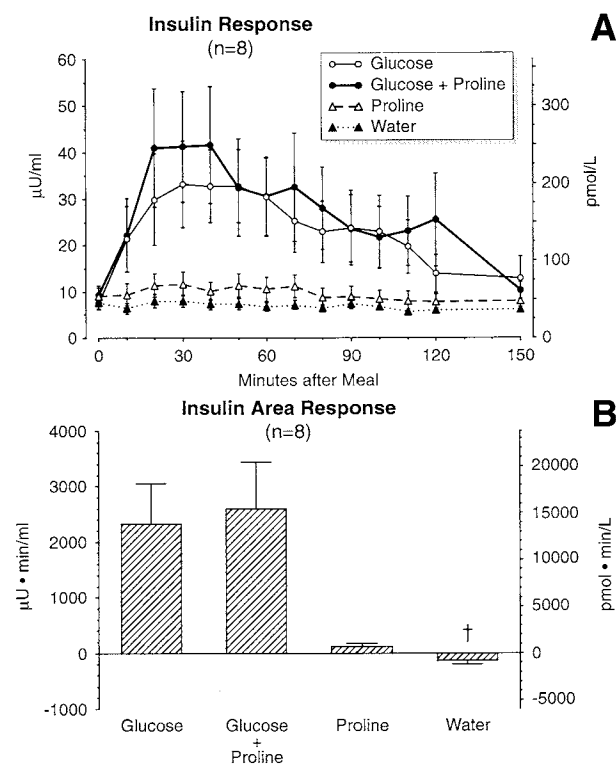
Fig 3. (A) Mean (± SEM) serum insulin concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. †Significantly different from proline alone using Student's *t* test (*P* < .02).
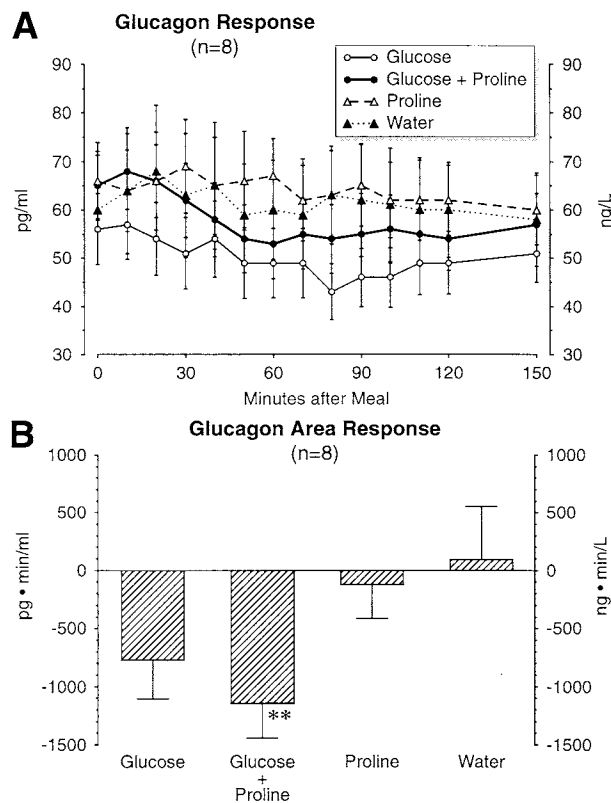
Fig 4. (A) Mean (± SEM) plasma glucagon concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Wilcoxon's sign rank test.
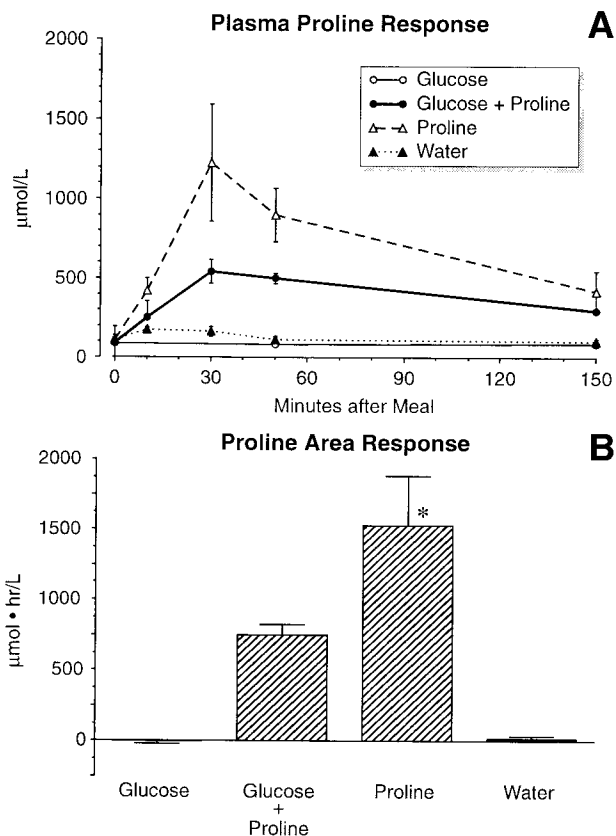

Fig 5. Mean (± SEM) plasma AAN concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Student's t test (P < .001); ††significantly different from glucose alone using Student's t test (P < .02).

and glucose alone compared with when they ingested water only (P < .05). However, the difference in fullness was not significantly different when the subjects ingested glucose with or without proline. Nevertheless, on average, they ate more of the mixed meal after proline was ingested (797 ± 80 kcal v 642 ± 81 kcal) than after water alone, but this was not statistically significant. After ingestion of glucose alone and glucose plus proline, subjects ingested 555 ± 68 kcal and 635 ± 53 kcal, respectively. Again, the subjects ingested more after proline ingestion. These differences also were not significant (P = .23).

The subjects found the sandy, grainy texture of the proline unpleasant, and a few described a bitter or sour taste. The subjects did not experience any unpleasant sensations, an unpleasant after-taste, or constitutional or gastrointestinal symptoms after the test meals.

DISCUSSION

Proline in proteins is important because its structure does not allow it to be present in the formation of an a-helix in the amino acid chain.[11] Proline is quantitatively absorbed after digestion of proteins.[12] Indeed, in the present study the peripheral circulating proline concentration had increased by 10 minutes after

its ingestion, and the concentration had increased approximately 13-fold by 30 minutes. Absorbed proline is metabolized to glutamine, which can enter the tricarboxylic cycle and ultimately be converted to glucose.[11]

Table 1. Plasma Amino Acid Concentrations

Time	0	50 Minutes	150 Minutes
Isoleucine			
Water	61 ± 7	53 ± 4	57 ± 5
Glucose	65 ± 4	43 ± 5*	39 ± 2*
Proline	56 ± 4	50 ± 3	51 ± 4
Pro + gluc	62 ± 5	49 ± 7	34 ± 5*
Leucine			
Water	118 ± 6	117 ± 10	112 ± 4
Glucose	127 ± 5	95 ± 5*	89 ± 4*
Proline	114 ± 8	105 ± 8	104 ± 4
Pro + gluc	132 ± 10	100 ± 12	74 ± 11*
Valine			
Water	211 ± 12	183 ± 7	199 ± 16
Glucose	162 ± 39	128 ± 33	129 ± 31
Proline	184 ± 15	168 ± 6	179 ± 13
Pro + gluc	219 ± 22	198 ± 22	154 ± 20

*Statistically significant compared with 0 time (ANOVA).

Table 2. Satiety Index and Caloric Consumption

	Glucose	Glucose + Proline	Proline	Water	Significance
Desire to eat	64 ± 6	58 ± 8	62 ± 8	71 ± 7	¥¥∧
Degree of hunger	59 ± 8	57 ± 8	59 ± 7	70 ± 6	†† ¥¥∧
Fullness	33 ± 7	42 ± 9	36 ± 8	24 ± 5	†¥∧
Proposed intake	62 ± 6	63 ± 8	68 ± 7	70 ± 7	†¥
Test meal taste	58 ± 8	47 ± 11	46 ± 11	59 ± 6	∧∧
Caloric intake (kcal)	563 ± 84	635 ± 53	797 ± 80	642 ± 81	
Protein (g)	24 ± 5	24 ± 4	28 ± 6	24 ± 3	
CHO (g)	67 ± 15	96 ± 11	117 ± 10	85 ± 19	ΔΔ
Fat (g)	23 ± 6	19 ± 4	27 ± 4	25 ± 5	

Statisical Significance Denoted by the Following Symbols		
	Rank Sum	P Value and Rank Sum
Glucose *v* glucose + proline	*	**
Glucose *v* proline	Δ	ΔΔ
Glucose *v* water	†	††
Glucose + Pro *v* proline	√	√√
Glucose + Proline *v* water	¥	¥¥
Proline *v* water	∧	∧∧

NOTE. Average response on a scale of 1-100 with 1 being the least and 100 the most. See Materials and Methods for more details.

Desire to eat: Glucose + proline is significantly less than glucose. Proline is significantly less than water (rank sum only).

Degree of hunger: Glucose, glucose and proline are significantly less than water. Proline is significantly less than water (rank sum only).

Fullness: Glucose, glucose + proline and proline are significantly higher than water (rank sum only).

Proposed intake: Glucose, glucose + proline are significantly less than water (rank sum only).

Test meal taste: Proline is significantly less than water.

CHO intake: Glucose is significantly less than proline.

The proline content of meats is approximately 4% to 5% on a molar basis. In cottage cheese, it is approximately 10%.[12] Thus, the amount of proline ingested by the subjects in this study was the equivalent of that found in 0.38 pounds (6 ounces) of beef or 0.15 pounds (2.4 ounces) of cottage cheese. These represent readily attainable amounts of proline ingested in a single meal. In the collagen family of proteins, another dietary source of protein, proline and its hydroxylated derivative, hydroxyproline, make up approximately 20% to 24% of the total number of amino acids present.

We were particularly interested in the effect of proline on circulating insulin and glucagon concentrations, because we previously had shown that ingested gelatin, the hydrolyzed product of collagen, strongly potentiated a glucose-stimulated increase in insulin (~270%) and reduced the plasma glucose response by approximately 30% in people with type 2 diabetes.[3] We were surprised by these results because the amino acid composition was considerably different from that of the other proteins studied.

Glycine, proline, and hydroxyproline make up approximately 55% to 65% of the total amino acids present in collagen, thus, we assumed that these amino acids contributed to the greatly increased insulin and smaller glucose response in people with type 2 diabetes when gelatin was ingested with glucose compared with when glucose was ingested alone.[3] However, neither glycine nor proline had been reported to stimulate insulin secretion.

Using the same protocol as used here, we previously have reported that glycine, the other major amino acid present in collagen, reduced the plasma glucose area response to ingested glucose by greater than 50% without a difference in insulin area response in normal subjects. This suggests that glycine stimulated insulin secretion in the presence of an increased glucose concentration. When glycine was ingested alone, it also stimulated a small increase in insulin concentration. Glycine ingestion also strongly stimulated an increase in glucagon concentration.[6]

The present data indicate that proline derived from the digestion of gelatin also is likely to have contributed to the smaller glucose response noted previously in the diabetic subjects when they ingested gelatin with glucose compared with when they ingested only glucose.[3] As with glycine, a proline-facilitated insulin response to glucose likely was playing a role. However, on a molar basis, glycine was more effective[6] than proline in reducing the glucose response. This occurred even though the quantitative insulin responses were similar.[6] A kinetic difference in response also was present. Proline stimulated an early increase in insulin, whereas, with glycine, the increase was slower than when glucose was ingested alone. An early increase in insulin, ie, a first phase insulin response is clearly important in maintaining a normal glucose concentration after a meal.[13,14]

To our knowledge, this is the first report of an effect on glucose and insulin metabolism by proline. In addition, based on the amount ingested, the effect is likely to be physiologically relevant.

The glucagon responses to glycine and proline clearly were different. Glycine stimulated an increase in concentration; proline did not. In fact, it resulted in a further decrease in glucagon concentration when it was ingested with glucose (Fig 3). Thus, glycine, but not proline, could have contributed to the robust

increase in glucagon concentration observed after gelatin ingestion.[3]

Of interest from a mechanistic perspective, the attenuation in glucose response was greater after glycine ingestion with glucose than after proline ingestion with glucose even though glycine and not proline stimulated an increase in glucagon concentration. This is contrary to the traditional concept of glucagon and insulin having opposing effects on glucose production in the liver.[15]

Another unexpected finding was that when glucose was ingested with proline, it greatly reduced the increase in proline concentration observed when proline was ingested alone (Fig 1). This must have been due to a decreased absorption rate or increased removal rate or both. The present data do not allow us to choose between these possibilities. Because glucose, when ingested alone, did not affect the proline concentration, most likely the simultaneous ingestion of glucose with the proline affected the absorption rate. Nevertheless, this remains to be documented. The decrease in leucine, isoleucine, and valine concentrations, which occurred after glucose or after glucose with proline ingestion, was as expected with an increase in insulin concentration.[16]

It also is of interest that proline, when ingested alone, decreased the subjects desire to eat and increased their sense of fullness (Table 2). However, on average, they ate more after the study than when they ingested water, glucose, or glucose with proline. The explanation for this is uncertain.

In the future, it will be of interest to determine if the hydroxylated prolines affect the circulating glucose, insulin, or glucagon concentrations. It also will be of interest to determine if there is an additive or even a synergistic effect on blood glucose and insulin concentrations when proline and glycine are ingested simultaneously in the same molar amounts. In addition, whether proline ingestion will reduce the glycine-stimulated increase in glucagon just as glucose did,[6] will be of interest. Whether the effect of the 2 amino acids can explain the additional stimulation of insulin secretion when gelatin was ingested with glucose by people with untreated type 2 diabetes[3] also will be of interest to determine. Finally, it will be of interest to determine if proline stimulates food ingestion significantly when a larger number of subjects are studied and when the amount of proline ingested is varied.

ACKNOWLEDGMENT

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Current Research

Glycemic and Insulinemic Responses to Protein Supplements

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ABSTRACT

Objective The effects of common servings of commercially marketed nutritional protein supplements on blood glucose and insulin responses were studied in 12 healthy men after ingestion of feedings that had varying carbohydrate and protein compositions.

Design Fasting subjects consumed a 50-gram glucose drink, a white bagel, peanuts, a protein bar, or a protein drink in a counterbalanced fashion.

Setting Subjects rested in a supine position and were not disturbed while blood samples were drawn at rest and at 10-minute intervals during the ensuing 2 hours.

Results The area under the curve for glucose was greater in the glucose drink group vs all treatment groups except the white bagel group ($P<.05$). At 20 to 40 minutes, plasma glucose was elevated in the glucose drink group vs the peanuts group, the protein bar group, and the protein drink group ($P<.05$). The glycemic response was greater in the glucose drink group vs the white bagel group at 30 minutes (8.1 ± 0.5 vs 6.5 ± 0.3 mmol/L, respectively) ($P<.05$). The area under the curve for insulin was lower in the peanuts group vs all treatment groups ($P<.05$). Insulin concentrations peaked at 40 minutes in the glucose drink group (285.5 ± 18.3 pmol) and was similar in all but the peanuts group (130.5 ± 14.3 pmol) ($P<.05$).

Conclusions A common serving of a commercially available protein supplement resulted in a marked insulin response with no glycemic response because of the lack of carbohydrate content. Inasmuch as many such supplements similar in composition are marketed on the bases of their nutritional energy benefits, these data underscore the need to educate consumers regarding appropriate fuel for exercise and nutritional supplement composition.
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Proper nutritional practices influence our general health and physical performance. Carbohydrate is a primary fuel source for the body, and adequate consumption is imperative to support prolonged physical activity (1,2). Intake of carbohydrate during exercise enhances endurance exercise performance (3); however, research examining pre-exercise carbohydrate feedings has shown that elevated insulin levels in response to the carbohydrate causes declines in blood glucose and inhibits lipid mobilization during subsequent exercise in some populations (4,5). Similarly, protein consumption has also been shown to elicit increases in plasma insulin concentrations (6,7).

To support or improve exercise performance, many individuals choose to supplement their diets with nutritional bars and drinks. Although composition and appropriate application of these supplements vary, most products are marketed as nutritional fuel support for exercise. Unfortunately, a protein nutritional bar or drink containing high amounts of protein and little carbohydrate would not provide fuel for exercise. To the contrary, the lack of carbohydrate and the potential insulin response may impair exercise performance.

Although glycemic index lists are quite comprehensive with regard to foodstuffs (8,9), limited data exist with regard to the glycemic and insulinemic responses to common servings of many food supplements, such as protein nutritional bars and drinks (10). We have evaluated the glucose and insulin response to a 50-g glucose feeding, a high–glycemic-index food (white bagel) and a known low–glycemic-index food (peanuts), and a solid (protein bar) and a liquid protein (protein drink) supplement. Based on previous literature, we hypothesized that common servings of protein supplements would return insulin responses similar to those from high-carbohydrate intake and, furthermore, that these metabolic responses would clearly show the lack of carbohydrate availability, thus contradicting the energy content claims made by many protein supplement manufacturers.

METHODS

Subjects

Twelve healthy, nondiabetic male subjects from the local college population participated in the study (Table 1). The University Human Subjects Institutional Review Board approved this study. All participants were informed regarding the purposes of the research and gave written informed consent before participation in the experimental trials.

Table 1. Physiological characteristics of subjects surveyed for glycemic and insulinemic responses to protein supplements

Age	27±1 y
Weight	75±3 kg
Body fat	14±3 %

Table 2. Nutrient content of feedings for subjects surveyed for glycemic and insulinemic responses to protein supplements

	Carbohydrate (g)	Protein (g)	Fat (g)
50 g glucose	50	0	0
Bagel	49	7	2.5
Peanuts	6	8	14
Solid protein bar	3	29	6
Liquid protein drink	2	15	4

Physiological Characteristics

On the first visit to the laboratory, the subject's height, weight, and body composition were measured. After voiding of urine, subjects were weighed without shoes and wearing shorts and a t-shirt. Body composition was measured using air-displacement plethysmography.

Experimental Trials

Subjects reported to the laboratory on five separate occasions, each separated by at least 72 hours. Subjects were in a 12-hour fasted state and had not participated in strenuous exercise during the preceding 24 hours. Subjects were instructed by senior researchers to keep a detailed 24-hour dietary record and to repeat this diet before each of the successive experimental trials. Dietary control for 24 hours before experimental trials in addition to the 12-hour fast were performed to attempt to further reduce basal blood metabolite variations within subjects. Because we were more concerned with diet duplication than diet composition, no analyses were performed on the subjects' pretrial food consumption.

On reporting to the laboratory, a 20-g flexible Teflon catheter was inserted into a forearm vein. The catheter was kept patent with a 0.9% sterile saline drip (≤1 mL/min). A resting blood sample was drawn. After the first blood sample, the subject consumed the prescribed feeding. Feedings were assigned in a counterbalanced fashion, and subjects were allowed 2 minutes to consume the feeding. Subsequent blood samples (3 mL) were drawn at 10, 20, 30, 40, 50, 60, 75, 90, and 120 minutes. During the testing period, subjects rested quietly in the supine position. Subjects were not allowed to sleep during the treatments or to rise from the examination table until after the last blood sample was collected. Standard lighting and a curtain drawn around the examination table minimized external stimuli.

Blood Analyses

Blood samples were stored in test tubes and kept in an ice slurry throughout experimental trials. After the trial, blood samples were centrifuged and the plasma was separated and stored at −80°C until analysis. Plasma glucose concentrations were analyzed in triplicate using spectrophotometric techniques with prepared kits (Sigma, St Louis, MO), and a human insulin-specific enzyme-linked immunosorbent assay (Crystal Chem, Chicago, IL) was used to assess plasma insulin concentrations.

Nutritional Treatments

In an attempt to mimic the likely food intake of consumers, serving design took into account an anticipated common serving based on packaging, energy content, and total feeding volume (Table 2). Total energy content for glucose in the glucose drink, white bagel, peanuts, protein bar, and protein drink were 200, 240, 240, 250, and 220 kcal, respectively. The glucose drink trial was included to provide a benchmark glucose and insulin response for comparison. This feeding design prevented establishing a glycemic index for any of the foodstuffs, but allowed for examining metabolic responses to normal food intake.

Statistical Analyses

The study used a factorial 5×10 design with repeated measures on the second factor. The first factor was nutritional treatment, and the second was time. The response variables were glucose and insulin concentrations. SAS proc mixed was used to test for main effects, interactions, and simple effects. In addition, total concentration of glucose and insulin in time (area under the curve [AUC]) for each nutritional treatment was calculated, and results were compared using a one-way analysis of variance. In the event of significant effects, a Tukey post-hoc analysis was used to make pair-wise comparisons to locate significant differences. The level of statistical significance was set at $P \leq .05$. All values are reported as mean±standard error.

RESULTS

Glucose Response

In the glucose drink group, plasma glucose concentrations were elevated above baseline at 10, 20, 30, and 40 minutes (Figure 1). They returned to baseline levels and were not different from fasting levels from 50 to 120 minutes. Plasma glucose values in the white bagel group were elevated above baseline only at 30 and 40 minutes after the feeding. Similar to the glucose drink group, plasma glucose levels declined to baseline at 50 minutes and remained there through 120 minutes. In all other trials, plasma glucose concentrations did not deviate from baseline levels during the entire sampling period.

When comparing glucose values between trials, significant elevations were seen in the glucose drink group at 20, 30, 40, and 50 minutes vs the peanuts group, the protein bar group, and the protein drink group (Figure 1). Likewise, blood samples during the white bagel trial showed higher glucose levels when compared with the protein drink trial at 40, 50, and 60 minutes. AUC for the glucose drink trial was greater than for all trials except the white bagel trial ($P = .08$) (Table 3).
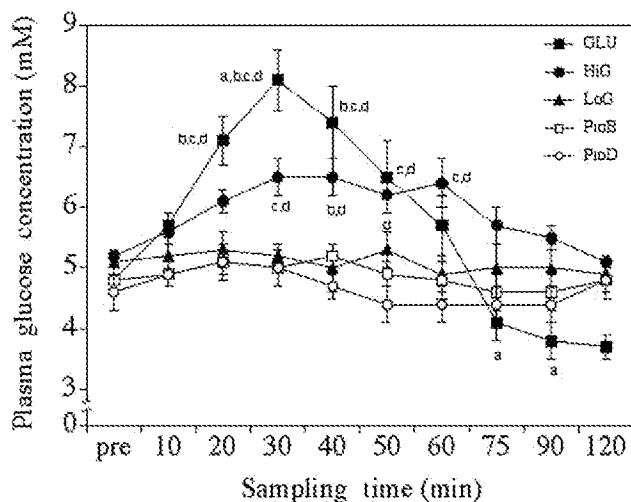
Figure 1. Plasma glucose concentrations over a 2-hour period after various feedings: a, different from the white bagel group; b, different from the peanuts group; c, different from the protein bar group; d, different from the protein drink group. $P \leq .05$. GLU, 50 g glucose; HiG, bagel; LoG, peanuts; ProB, solid protein bar; ProD, liquid protein drink.

Figure 2. Plasma insulin concentrations over a 2-hour period after various feedings: a, different from the glucose drink group; b, different from the white bagel group; c, different from the protein bar group; d, different from the protein drink group. $P \leq .05$. GLU, 50 g glucose; HiG, bagel; LoG, peanuts; ProB, solid protein bar; ProD, liquid protein drink.

Table 3. Area under the curve for plasma glucose and insulin in subjects taking protein supplements[a]

Feeding	Plasma glucose	Plasma insulin
50 g glucose	100	100
Bagel	61	91
Peanuts	3[b]	57[b]
Solid protein bar	1[b]	90
Liquid protein drink	10[b]	88

[a]Values are percents of glucose.
[b]Less than glucose, $P<.05$

Insulin Response

The glucose drink group, the protein bar group, and the protein drink group plasma insulin concentrations were elevated above baseline from 10 through 60 minutes ($P<.05$) (Figure 2). Insulin values in the white bagel group exceeded baseline from 30 to 75 minutes, whereas there was no change in insulin concentrations in the peanuts group. Plasma insulin had returned to near baseline by 90 minutes in all trials.

By 20 minutes, plasma insulin values in the glucose drink group were significantly greater than in the white bagel group and the peanuts group, and remained greater than in the peanuts group through 60 minutes ($P<.05$). Insulin concentrations in the peanuts group were lower than those measured in the white bagel group, the protein bar group, and the protein drink group at various time points from 20 through 75 minutes (Figure 2). Analyses of insulin AUC showed that the glucose drink trial was similar to all trials with the exception of the peanuts trial (AUC=57% of the glucose drink group) (Table 3) ($P<.05$).

DISCUSSION

Insulin mediates glucose and amino acid uptake in the cells. When fed carbohydrate, normal healthy individuals will respond with increases in plasma glucose and a concomitant elevation in plasma insulin concentrations (1). Similarly, the ingestion of a protein-rich meal will elicit insulin release from the pancreas (6). This study compared the glycemic and insulinemic responses of four different feedings with a 50-g oral glucose load over a 2-hour period.

In our subjects, a normal glucose response was observed after consumption of feedings containing significant amounts of carbohydrate (Figure 1). Furthermore, significant increases in plasma insulin concentrations were measured in response to the carbohydrate and protein supplement treatments (Figure 2). Inasmuch as similar protein supplements on the market are labeled as energy bars and may be accompanied by suggestions for consumption before, during, and/or after exercise, these normal and expected metabolic responses should be considered when consuming such supplements.

Consumption of a liquid or solid carbohydrate feeding causes an increase in plasma glucose concentrations (1). While at rest, the body will regulate this elevation in glucose with the release of insulin to facilitate glucose uptake into the cells and return plasma glucose concentrations to homeostatic levels. The glycemic response in the glucose drink group is consistent with data presented in the scientific literature (Figure 1). Likewise, glucose AUC values for the white bagel and the peanuts trials are comparable with those seen in other normal, healthy subjects (Table 3). As was expected, the nutritional supplement trials containing low amounts of carbohydrate (peanuts, protein bar, and protein drink) showed glucose AUC values significantly lower than in the glucose drink trial.

Insulin is responsible not only for stimulating uptake of glucose, but also for the movement of amino acids from

the blood stream into the cells (11); hence, the secretion of insulin from the pancreas increases in response to oral carbohydrate and protein loads (7). In the present study, plasma insulin concentrations increased after all feedings with the exception of the peanuts (Figure 2). As expected, a rapid insulin response was seen in the glucose drink group. Likewise, the protein drink trial produced an insulin response similar to that in the glucose drink trial. The protein drink contained 30 g protein and only 8 g carbohydrate. This type of insulin response is consistent with that previously reported in the literature. In fact, the plasma insulin response to a 50-g carbohydrate load and a 50-g protein load have been shown to be similar (6).

It has been shown that when elevated insulin concentrations are present at the onset of exercise, there is an exponential increase in muscle glucose uptake. This is the result of the synergistic effect of insulin and contraction-mediated stimulation of glucose transport protein 4 translocation (12). This metabolic situation may result in sharp declines in blood glucose concentrations to near hypoglycemic levels at the beginning of exercise, and has been implicated in reductions in endurance exercise performance (5,13). The protein supplements studied in this investigation, if consumed before exercise, would likely cause an increase in plasma insulin and possibly elicit exceptionally low blood glucose values during the early stages of exercise. This potential metabolic impact is noteworthy for consumers.

Product packaging and the accompanying nutritional information influences consumers' beliefs and choices (14-16). Information contained on nutritional supplement packaging puts forth the concepts of sports and energy, whether overtly stated or subtly implied. Many nutritional supplements may use generic terms such as "sports" or "energy" to describe the food product; however, interpretation of these descriptors is left to the consumer (17). Consumers seeking nutritional support for endurance performance would be unwise to select a supplement largely intended to provide additional protein to support muscular adaptation. In fact, there is real potential for ergolytic effects.

CONCLUSION

Limitations of this study include small sample size, thereby affecting generalizability of results. Also, only male participants were involved, and they were not physically active. Impaired glucose tolerance was not addressed, but remains an important consideration in these studies.

In summary, the findings of this investigation show that insulinemic responses to consuming ~30 g protein in a liquid or solid nutritional supplement are similar to those after a 50-g oral glucose load. Although the utility of nutritional bars and drinks to provide additional protein in the diet is not questioned, the intake of supplements such as these when marketed as a sport or energy supplement may have negative implications for exercise performance for the uninformed consumer. These data support the need for increased accuracy in product packaging and marketing, as well as consumer education to encourage appropriate selection of nutritional supplements.

Individuals may choose to supplement their diets with nutritional bars and drinks in an attempt to support the energy demands of exercise or to enhance physiologic adaptation. An understanding of the composition and subsequent metabolic effects of nutritional supplements will make an individual a more critical and competent consumer. The current data are intended to assist nutrition professionals when educating individuals regarding nutritional supplement selection and application, specifically, that lay individuals may be able to determine the appropriate application of a nutritional supplement on the bases of actual nutrient content, as opposed to product packaging descriptions.

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APPLICATIONS

Feeding the Athlete

Competitive and recreational athletes are always looking for ways to improve their performance. Individualizing a plan of adequate fuel, fluids, and nutrients to assist them in doing their best can be a challenging task for dietetics professionals. The use of sport supplements and their perceived value by the athlete may increase this challenge. In a recent survey of university student athletes, Burns and colleagues showed that 88% used one or more nutritional supplements (1). With thousands of products available in stores or on the Internet, questioning the athlete as to the type of supplement used and its specific nutrients is a must for an informed counseling session. All nutrition recommendations for athletes should be based on current scientific data and the needs of athletes as individuals (2).

In the previous article, researchers Parcell and colleagues studied the effects of five different food items on 12 male college students. The glycemic and insulin response to glucose, a bagel, peanuts, and both protein drink and a protein bar supplement were then monitored. The peanuts and protein supplements, which were lowest in carbohydrates, performed as expected, with significantly lower glycemic response than the glucose or bagel. Of note, plasma insulin concentrations rose following all feedings except that of the peanuts. The authors state that this type of insulin response on normoinsulinemic subjects is consistent with studies previously reported (3). This insulin response is also seen in persons with controlled type 2 diabetes. As presented in Franz and colleagues, "ingested protein does not increase plasma glucose concentrations, although protein is just as potent a stimulant of insulin secretion as carbohydrate" (4).

This research reinforces the need for educating our clients on the types of supplements they are taking—whether they are sports, herbal, vitamin, or low-carbohydrate supplements—and giving them informed choices on continuing usage based on their desired outcome. Dietetics professionals must encourage patients to bring in actual labels of supplements they are taking. This will allow the focus to be

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placed on the ingredient list and Nutrition Facts label and not on the promised advertising or label hype.

Athletes need to adopt a healthful diet to achieve maximum performance. For dietetics professionals working with a variety of patients, such as those in an outpatient general practice, the knowledge needed to work with athletes may be considered skills only required by those specializing in sports nutrition. Basic sports nutrition knowledge is vital because all clients—pediatric or adult—should be encouraged to be "athletes" and exercise as part of a healthful lifestyle. Sports nutrition continuing education should be included in all portfolios as needed. There is a unique resource called the Performance Challenge Program available to assist dietetics professionals in delivering updated information on nutrition, hydration, and supplements. This is a no-cost program available through the American Dietetic Association (ADA) Web site (www.eatright.org). Other ADA resources for enhancing sports professional development exist through the Sports, Cardiovascular, and Wellness Nutritionists (SCAN) dietetic practice group (www.scandpg. org) or *Sports Nutrition: A Guide for the Professional Working with Active People* (3rd edition, 1999), available through the ADA.

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Role of Amino Acids in Stimulation of Postprandial Insulin, Glucagon, and Pancreatic Polypeptide in Humans

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Summary: Protein-rich meals stimulate secretion of insulin, glucagon, and pancreatic polypeptide (PP) from the endocrine pancreas. On the one hand, this is due to increased levels of circulating amino acids, and, on the other, neural and/or endocrine factors can contribute to activation of islet cell function. The present study was designed to determine, first, pancreatic endocrine function and postprandial amino acid levels after a protein and a protein-carbohydrate meal and second, insulin, glucagon, and PP levels during infusion of amino acid mixtures that imitate the postprandial amino acid pattern. In healthy volunteers the ingestion of a protein-rich meal (300 g tenderloin steak) elicited within 1 h an increase of virtually all amino acids by 20–400 μmol/L above basal values. The infusion of two different amino acid solutions available for use in humans showed that Aminosteril-N-Hepa (AS) was better for the imitation of the so-called "insulinogenic" amino acids while Aminoplasmal L-10 (AP) gave more comparable plasma levels of the "glucagonogenic" amino acids. Both solutions were not able to imitate the postprandial amino acid pattern completely. With regard to insulin levels, both solutions gave a comparable increase, while AP but not AS stimulated glucagon and PP levels. This suggests that circulating amino acids may be responsible for 60% of the postprandial insulin response after a protein meal, while their contribution to glucagon release can only be roughly estimated at 30–60%. The contribution of circulating nutrients to the greater insulin response after the protein-carbohydrate meal was comparable (60%), while the attenuated glucagon response can be ascribed almost completely to the effect of circulating nutrients. In conclusion, the present data demonstrate that the composition of amino acid mixtures is as yet not ideal for a complete imitation of the postprandial amino acid pattern. The insulin, glucagon, and PP response depends on the amino acid mixtures and accordingly the respective plasma amino acid concentrations obtained during infusion studies. The adequate imitation of plasma amino acid levels is of critical importance for the evaluation of absorbed and circulating amino acid effects in the postprandial state. **Key Words:** Insulin—Glucagon—Pancreatic polypeptide—Amino acid mixtures.

The ingestion of a mixed meal stimulates the release of insulin, glucagon, and pancreatic polypep-

tide (PP) from the islets of Langerhans. This postprandial increase of pancreatic endocrine function is due to the increase of intestinal hormone secretion in combination with the stimulatory action of increasing levels of circulating nutrients such as glucose and amino acids. For PP but not insulin release, cephalic mechanisms are also of substantial importance (1–4). Gut factors have been suggested

to augment not only the insulin response after carbohydrate meals (5–8) but also that following the ingestion of protein-rich meals (9) so that the insulin stimulatory action of absorbed and circulating amino acids (10) is increased by intestinal hormones.

Protein meals stimulate not only insulin but also glucagon release (11), and it has been shown that circulating amino acids and gastrointestinal hormones can act both separately and synergistically on glucagon release (12–15), although this has been the subject of some debate (16).

With regard to the effects of amino acids on pancreatic endocrine function, it has been shown that a large number of the various amino acids normally present in the circulation stimulate insulin and glucagon release in vitro (17–20) and also in vivo (10,12–15,21,22). In contrast to studies with glucose, the range of physiological perturbations of the various amino acids is virtually unknown. Therefore, it is difficult to decide if those effects observed during the infusion of fairly high doses of single amino acids or mixtures reflect physiological or pharmacological effects unless plasma levels of the respective amino acids are determined. This is important to consider since the various amino acids have a different stimulatory action on insulin (10,22) and glucagon (21) release when compared on an equimolar or weight basis. Thus, the relative increase of predominantly glucagon-stimulating amino acids such as asparagine, glycine, phenylalanine, serine, and aspartate (21) or, on the other hand, that of predominantly insulin-stimulating amino acids such as tryptophan, leucine, asparagine, isoleucine, and glutamine (10,21) could lead to a misinterpretation of the role that amino acids play as physiological stimuli of postprandial insulin and glucagon release.

Therefore, the present study was designed to determine, first, insulin, glucagon, PP, and postprandial amino acid levels after a protein or a protein-carbohydrate meal and second, insulin, glucagon, and PP levels during infusion of amino acid mixtures that imitate most adequately the postprandial amino acid pattern.

MATERIALS AND METHODS

Experimental subjects

The studies were performed in 14 healthy male subjects aged 19–35 years who were within 10% of ideal body weight. All subjects gave their informed written consent prior to the experiments.

Ingestion of test meals

After an overnight fast, nine subjects ingested within 15 min a protein-rich meal consisting of 300 g tenderloin pork meat (protein-rich meal) together with 200 ml of water. Blood samples were obtained from a forearm vein via an indwelling catheter before and at various time points thereafter for 240 min. On another day the same individuals ingested a meal consisting of 300 g tenderloin pork meat and 50 g of bread together with 20 g glucose dissolved in 200 ml of water (protein-carbohydrate meal). The protein and fat content of the meat meal was 54 and 30 g, respectively.

Intravenous infusion studies

After an overnight fast, the subjects received on separate days in randomized order with at least a 1-day interval infusions of saline (2 ml/min), glucose (0.2 g/min), mixed amino acids [Aminoplasmal L-10 10% (AP), 2.0 ml/min, or Aminosteril-N-Hepa 8% (AS), 2.5 ml/min], or a combination thereof. The composition of the amino acid solutions is shown in Table 1.

The following test protocols were examined: saline, glucose, AP, AS, AP + glucose, and AS + glucose.

Not all the test protocols were performed in the same subjects. However, care was taken that the test protocols necessary for a direct comparison were performed in the same individual. The number of subjects receiving the same infusion protocols required for an intraindividual comparison of the effects is indicated in the text and in the legends to the figures.

TABLE 1. Amino acid composition of the two test solutions employed

	Aminoplasmal L-10 (g/L)	Aminosteril-N-Hepa 8% (g/L)
Lysine	7.00	9.71
Valine	4.80	10.08
Leucine	8.90	13.08
Arginine	9.20	10.72
Isoleucine	5.10	10.40
Threonine	4.10	4.40
Proline	8.90	5.73
Alanine	13.70	4.64
Glutamic acid	4.60	—
Tryptophan	1.80	0.70
Ornithine-HCl	3.20	—
Serine	2.40	2.24
n-Acetyl-tyrosine	1.23	—
Tyrosine	0.30	—
Histidine	5.20	2.80
Methionine	3.80	1.10
Phenylalanine	5.10	0.88
Glycine	7.90	5.82
Aspartic acid	1.30	—
Cysteine-HCl	0.73	0.52

Blood sampling and hormone assay

Blood samples were obtained from a contralateral forearm vein via an indwelling catheter and collected into tubes containing 3 mg EDTA and 1,000 kIU Trasylol. All samples were kept chilled in an ice bath until centrifugation at 2,000 rpm for 15 min at 4°C. The separated plasma was stored at -20°C until time of assay.

Plasma insulin (23) and glucagon (24) were determined as described elsewhere. Antibody 30K for glucagon measurements was generously provided by Dr. R. H. Unger (Dallas, TX, U.S.A.). PP levels were determined as described previously (25). Standard human pancreatic polypeptide (hPP) and rabbit anti-hPP serum were a generous gift from R. E. Chance (Lilly Research Lab., Indianapolis, IN, U.S.A.).

For statistical comparison between the mean of the baseline values and subsequent time points during stimulation by food or intravenous infusions, analysis of variance for multiple determination was employed and p values of ≤ 0.05 were considered significant.

RESULTS

Effect of a protein-rich meal

In nine subjects the ingestion of the protein-rich test meal elicited within 30–45 min a significant increase of the plasma levels of all 21 amino acids measured in the present study (Table 2). Most amino acids remained elevated for the entire experimental period of 240 min. The hormonal response to the test meal is shown in Fig. 1. Insulin rose from a mean basal level of 5 μU/ml to a peak level of 16

TABLE 2. *Effect of a protein-rich test meal consisting of 300 g tenderloin pork meat on plasma amino acid concentrations (μmol/L) in nine subjects (means ± SEM)*

Amino acid	\-20	\-10	0	15	30	45	60	75	90	105	120	150	180	210	240
Lysine	191	191	178	208	256	347	349	472	506	548	554	595	557	346	352
	± 26	± 30	± 25	± 32	± 48	± 78	± 69	± 116	± 117	± 115	± 101	± 119	± 97	± 22	± 23
Leucine	152	158	147	154	169	253	267	340	380	418	403	507	494	372	400
	± 13	± 18	± 14	± 17	± 22	± 54	± 51	± 67	± 77	± 59	± 67	± 79	± 61	± 34	± 38
Valine	256	253	250	266	298	358	378	426	456	489	543	603	600	453	500
	± 28	± 25	± 26	± 28	± 41	± 55	± 54	± 73	± 80	± 61	± 76	± 82	± 73	± 35	± 42
Isoleucine	86	77	79	79	100	136	150	194	214	244	271	297	293	229	257
	± 7	± 6	± 6	± 6	± 12	± 23	± 26	± 33	± 39	± 34	± 34	± 39	± 29	± 21	± 27
Proline	178	174	175	240	266	271	298	290	258	287	338	373	288	328	189
	± 20	± 11	± 18	± 25	± 23	± 26	± 22	± 24	± 24	± 28	± 22	± 32	± 27	± 31	± 28
Arginine	96	88	93	103	132	162	195	228	251	261	274	289	264	188	194
	± 11	± 8	± 10	± 13	± 22	± 40	± 29	± 47	± 55	± 39	± 44	± 49	± 36	± 19	± 16
Glutamine	367	438	460	525	565	595	599	616	616	623	624	625	622	447	453
	± 97	± 89	± 85	± 75	± 83	± 90	± 89	± 86	± 84	± 83	± 82	± 81	± 79	± 23	± 24
Threonine	142	144	146	175	205	289	216	243	276	278	299	299	286	209	193
	± 16	± 20	± 21	± 40	± 52	± 87	± 33	± 38	± 38	± 31	± 40	± 36	± 31	± 16	± 13
Alanine	360	359	361	370	433	480	486	508	489	507	499	489	455	358	403
	± 30	± 34	± 34	± 25	± 42	± 57	± 45	± 47	± 40	± 40	± 39	± 33	± 32	± 22	± 49
Taurine	109	102	109	136	148	170	174	185	192	177	168	155	179	212	237
	± 23	± 18	± 16	± 22	± 18	± 25	± 21	± 25	± 31	± 26	± 17	± 18	± 28	± 26	± 19
Glutamic acid	67	65	65	99	112	123	131	147	166	158	179	159	157	87	98
	± 11	± 18	± 17	± 40	± 34	± 38	± 36	± 32	± 30	± 35	± 44	± 27	± 34	± 9	± 9
Tryptophan	54	60	66	65	69	80	81	93	103	104	115	149	125	74	79
	± 13	± 15	± 16	± 15	± 17	± 22	± 20	± 25	± 29	± 26	± 27	± 41	± 25	± 13	± 15
Histidine	83	80	80	84	109	147	140	162	163	168	163	163	145	100	100
	± 10	± 9	± 11	± 12	± 18	± 26	± 21	± 28	± 28	± 24	± 24	± 26	± 21	± 6	± 8
Glycine	258	242	266	274	304	325	338	342	337	332	322	314	288	220	246
	± 19	± 23	± 29	± 33	± 37	± 52	± 38	± 33	± 36	± 23	± 28	± 24	± 21	± 18	± 38
Serine	119	120	122	131	146	169	165	181	191	195	206	197	187	140	136
	± 11	± 11	± 14	± 15	± 21	± 29	± 18	± 27	± 28	± 19	± 26	± 11	± 17	± 10	± 13
Tyrosine	78	83	81	79	86	102	92	117	124	151	157	165	163	127	138
	± 6	± 7	± 12	± 8	± 10	± 14	± 16	± 19	± 22	± 27	± 19	± 21	± 18	± 17	± 18
Ornithine	76	81	60	67	85	109	93	119	119	134	143	160	160	113	121
	± 10	± 13	± 10	± 11	± 12	± 18	± 14	± 26	± 21	± 23	± 25	± 26	± 25	± 8	± 14
Methionine	35	30	33	30	39	58	65	78	72	69	86	95	90	78	78
	± 4	± 2	± 3	± 5	± 2	± 8	± 12	± 12	± 12	± 19	± 10	± 10	± 8	± 9	± 12
Asparagine	50	52	51	50	58	72	81	97	101	158	179	159	157	87	98
	± 5	± 4	± 6	± 5	± 7	± 13	± 12	± 13	± 16	± 35	± 44	± 27	± 34	± 9	± 9
Phenylalanine	50	56	55	55	51	74	71	84	83	102	89	103	98	78	79
	± 7	± 5	± 6	± 6	± 8	± 11	± 10	± 11	± 12	± 18	± 14	± 12	± 10	± 11	± 11
Aspartic acid	7	6	6	9	9	12	15	16	23	20	24	23	25	26	22
	± 1	± 1	± 1	± 1	± 1	± 2	± 2	± 3	± 3	± 2	± 3	± 9	± 8	± 5	± 5

FIG. 1.   Effect of a protein-rich or a protein-carbohydrate meal on peripheral vein plasma insulin (IRI), glucagon (IRG), pancreatic polypeptide (PP), and glucose levels in nine subjects (means ± SEM).

± 3 μU/ml within 60 min, decreasing thereafter toward baseline. The incremental insulin response was 76.5 ± 8 μU/240 min. Glucagon levels increased from 270 ± 30 to 550 ± 60 pg/ml at 90 min, remaining at this plateau until the end of the experiment. The incremental glucagon response was 2,609 ± 274 pg/240 min. PP was released from a baseline of 45 ± 10 to 300 ± 25 pg/ml within 15 min, decreasing thereafter slightly by 100 pg/ml and increasing again to a plateau of 325 pg/ml for the rest of the observation period. The incremental PP response was 2,857 ± 270 pg/240 min. Plasma glucose levels remained between 3.9 and 4.2 mmol/L (Fig. 1).

Effect of intravenous amino acids on plasma amino acid levels

In eight subjects two different amino acid mixtures were applied. With each solution a different pattern of circulating amino acids was obtained (Fig. 2).

During the infusion of AP at 2 ml/min, it was not possible to reach the postprandial levels of lysine, leucine, valine, isoleucine, taurine, glutamic acid, tryptophan, or tyrosine, while proline, arginine, glutamine, threonine, histidine, serine, ornithine, methionine, asparagine, phenylalanine, and aspartic acid were in the range of the postprandial levels (Fig. 2). Alanine and glycine, however, were approximately twice the postprandial levels. With the other infusion mixture (AS), postprandial amino acid levels of leucine, valine, isoleucine, proline, arginine, threonine, and alanine were well imitated. With the exception of glycine, which was too high, all the other amino acids were below the postprandial range (Fig. 2).

Effect of intravenous amino acids on hormone levels

AP stimulated plasma insulin from 8 ± 0.5 μU/ml to a peak of 12 ± 1 μU/ml within 20 min after onset of the infusion (Fig. 3). Glucagon levels increased from 195 ± 15 to 325 ± 32 pg/ml within 80 min (p < 0.01) and remained elevated until the end of the experiment. PP levels rose significantly from 70 ± 8 pg/ml to a maximum of 160 ± 20 pg/ml at the end of the infusion period (p < 0.01). Plasma glucose levels were constant at 4.5 mmol/L throughout the infusion period (Fig. 3). During the infusion of AS, insulin rose from a mean basal value of 9.5 ± 0.7 μU/ml to a peak of 16 ± 2 μU/ml at 20 min. Basal glucagon levels averaged 205 ± 25 pg/ml and increased to 275 ± 25 pg/ml at 60 min (p < 0.05). PP levels showed a small increase from 85 ± 9 to 130 ± 20 pg/ml. This increase, however, was not statistically significant. Plasma glucose levels remained at 4.3 mmol/L (Fig. 3). While the effect of both amino acid solutions was fairly similar on insulin levels, AP elicited a more pronounced elevation of glucagon and PP compared with the effect of AS. During the infusion of saline, there was no change of insulin, glucagon, PP, or glucose levels (Fig. 3).

Effect of a combined protein-carbohydrate meal

Nine subjects ingested a meal consisting of 300 g meat, a slice of bread (50 g), and 20 g glucose dissolved in 200 ml water. After ingestion of the meal, insulin rose from a mean basal value of 6 ± 0.5 μU/ml to a peak of 43 ± 5 μU/ml at 30 min, decreasing thereafter gradually to baseline values during the observation period (Fig. 1). The incremental insulin response was 166 ± 21 μU/240 min. Basal
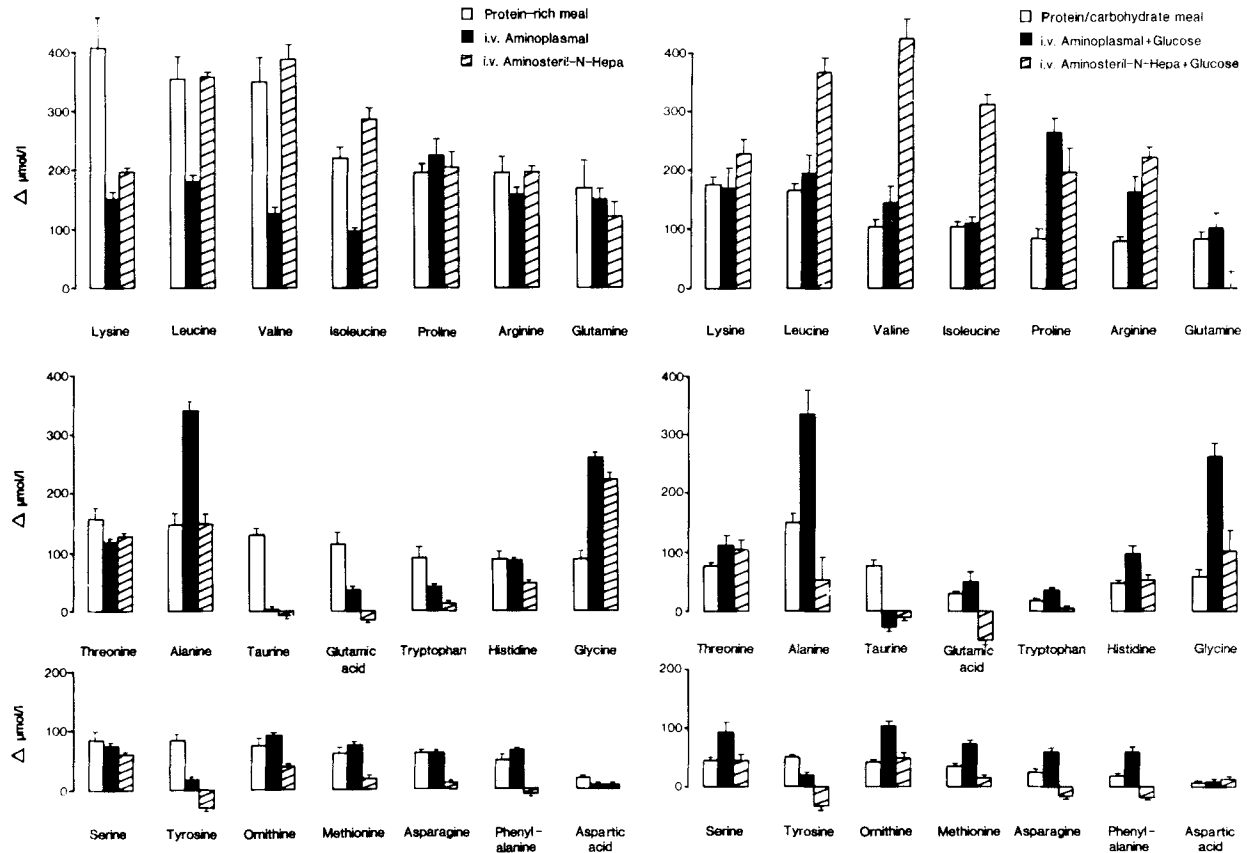
FIG. 2.   Maximal increase of peripheral vein plasma amino acid concentrations (μmol/L) above the mean baseline level following the ingestion of a protein-rich test meal or during intravenous infusion of Aminoplasmal L-10 or Aminosteril-N-Hepa (left) (means ± SEM) and maximal increase of peripheral vein plasma amino acid concentrations (μmol/L) above the mean baseline level following the ingestion of a protein-carbohydrate meal or during intravenous infusion of Aminoplasmal L-10 + glucose or Aminosteril-N-Hepa + glucose (right) (means ± SEM).

glucagon levels averaged 225 ± 12 pg/ml and rose slightly to only 350 ± 10 pg/ml at 180 min compared with the rise of glucagon by 300 pg/ml after the pure protein meal. The incremental glucagon response was 895 ± 103 pg/240 min. Plasma glucose levels increased within 30 min of ingestion of the test meal and reached a peak value of 5.5 ± 0.7 mmol/L. Plasma PP levels rose from a mean baseline of 60 ± 7 pg/ml to a maximum of 400 ± 60 pg/ml at 30 min (Fig. 1). The incremental PP response was 3,214 ± 365 pg/240 min. The pattern of circulating amino acids changed after ingestion of the combined meal compared with protein alone. The rise of all the amino acids with the exception of alanine was considerably less as compared with the protein-rich meal (Table 3). Although absolute values were lower, the relative distribution of the amino acids remained fairly identical during the combined test meal (Table 3; Fig. 2).

Effect of intravenous amino acids on plasma amino acid levels during simultaneous glucose infusion

The infusion of AP + glucose resulted in a fairly good imitation of the postprandial levels of lysine, leucine, valine, isoleucine, glutamine, threonine, and aspartic and glutamic acid (Fig. 2). Other amino acids such as proline, arginine, alanine, histidine, glycine, serine, ornithine, methionine, asparagine, and phenylalanine were approximately twice the postprandial concentrations (Fig. 2).

The infusion of AS, which contains more branched-chain amino acids and which imitated the amino acid pattern of the pure protein meal fairly well, elicited a substantial elevation of several amino acids above the postprandial range. This includes leucine, valine, isoleucine, proline, arginine, and glycine (Fig. 2). Furthermore, there was a reduction or at least no elevation of glutamine, alanine, taurine, glutamic acid, tryptophan, tyrosine,
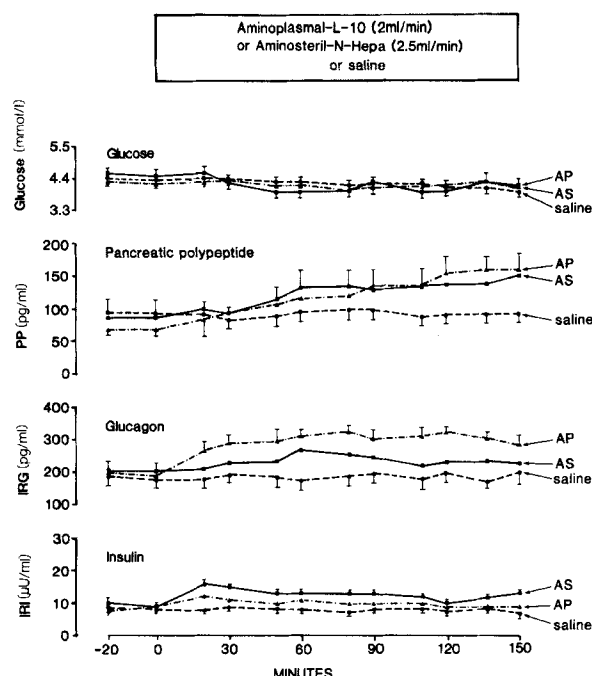
FIG. 3. Effect of an intravenous infusion of Aminoplasmal L-10 (AP), Aminosteril-N-Hepa (AS), or saline on peripheral vein plasma insulin (IRI), glucagon (IRG), pancreatic polypeptide (PP), and glucose levels in eight subjects (means ± SEM).

methionine, asparagine, and phenylalanine. It should be noted that these amino acids are either not at all present or present only in low concentrations in the amino acid solution employed (Table 1).

Effect of intravenous amino acids + glucose on hormone levels

The infusion of AP + glucose stimulated insulin levels from a basal value of 8 + 0.7 μU/ml by maximally 22 ± 2.5 μU/ml within 30 min (Fig. 4). In contrast, the increase of insulin during AS + glucose was only 16 ± 2 μU/ml. This difference of insulin levels at 30 min was statistically significant (p < 0.01) while all other insulin levels were identical. Nevertheless, both amino acid mixtures potentiated the insulin release induced by intravenous glucose alone (Fig. 4). During AP + glucose or AS + glucose, the rise of plasma glucagon levels was similar as compared with the respective amino acid infusions without glucose, except for the first 30-min period where glucagon levels were not yet significantly elevated during the combined infusions (Figs. 3 and 4). Glucose levels rose initially by 1.1 mmol/L during both infusion mixtures with a maximum at 30 min, decreasing thereafter toward baseline levels (Fig. 4). Glucose alone elicited a similar

maximal increase of plasma glucose levels at 30 min; thereafter, they were ~0.55 mmol/L higher compared with the combined infusion of amino acids + glucose (Fig. 4). PP levels rose significantly by 40 pg/ml (p < 0.01) during AP + glucose at 110 and 120 min.

During AS + glucose, there was no significant alteration of PP levels. The apparent increase as shown in Fig. 4 was due mainly to three experiments, while the others remained in the range of the baseline.

DISCUSSION

The effect of amino acids on insulin and glucagon secretion has been evaluated extensively in vitro and in vivo. Floyd et al. (10) have demonstrated large differences among amino acids in their capacity to stimulate insulin secretion in humans. Similarly, Rocha et al. (21) have examined the effect of single amino acids on glucagon secretion in dogs, showing that asparagine, glycine, phenylalanine, serine, and aspartate have the greatest effect on A-cell function when compared on a molar basis. In these and also in subsequent studies using amino acid mixtures to stimulate pancreatic endocrine function, plasma amino acid levels during infusion were not determined. Thus, circulating amino acid levels might have been beyond the range of physiological perturbations.

The present study compares for the first time postprandial amino acid concentrations during a protein meal with those obtained during infusion of amino acid mixtures that are available for use in humans. No commercially available amino acid mixture was suitable to imitate the postprandial changes of all amino acids. Considering the major insulinogenic amino acids tryptophan, leucine, asparagine, isoleucine, glutamine, and arginine, AS, which contains the branched-chain amino acids in higher concentrations, gave the better comparison for these amino acids than AP. It should be noted, though, that some amino acids such as tryptophan and asparagine were still considerably below the postprandial levels.

The infusion of AS had no significantly different effect on insulin secretion as compared with AP, although this might have been expected considering the amino acid composition. This might be due to the fact that two important insulinogenic amino acids (tryptophan, glutamine) are still too low during AS infusion and some others, although not that potent on β-cell activity, are missing completely. It

TABLE 3. *Effect of a protein-carbohydrate test meal on plasma amino acid concentrations (μmol/L) in nine subjects (means ± SEM)*

Amino acid	min														
	−20	−10	0	15	30	45	60	70	90	105	120	150	180	210	240
Lysine	138	140	150	148	188	195	239	257	285	289	319	312	311	288	297
	± 21	± 5	± 10	± 8	± 10	± 10	± 18	± 19	± 20	± 17	± 26	± 12	± 12	± 12	± 15
Leucine	132	123	139	156	159	159	177	196	225	238	261	295	284	279	273
	± 5	± 4	± 16	± 2	± 8	± 3	± 4	± 3	± 12	± 6	± 9	± 16	± 17	± 14	± 20
Valine	197	181	196	206	215	206	248	244	275	284	308	337	344	346	344
	± 7	± 7	± 18	± 33	± 14	± 8	± 23	± 23	± 15	± 9	± 8	± 15	± 18	± 18	± 21
Isoleucine	71	70	67	66	83	90	108	114	131	141	155	174	172	171	169
	± 6	± 5	± 4	± 4	± 4	± 3	± 5	± 4	± 7	± 5	± 6	± 7	± 10	± 6	± 12
Proline	156	158	163	168	241	208	243	234	251	223	222	234	227	242	206
	± 12	± 13	± 16	± 23	± 35	± 20	± 31	± 20	± 26	± 18	± 19	± 20	± 22	± 28	± 35
Arginine	68	67	65	66	90	90	101	111	130	132	143	147	141	135	128
	± 7	± 4	± 4	± 3	± 7	± 5	± 5	± 5	± 10	± 7	± 8	± 5	± 8	± 8	± 8
Glutamine	414	379	391	399	410	410	420	435	460	454	449	444	479	465	464
	± 23	± 17	± 14	± 21	± 18	± 15	± 37	± 18	± 20	± 14	± 16	± 21	± 20	± 22	± 22
Threonine	100	95	94	96	116	120	126	134	152	155	164	170	164	163	158
	± 6	± 6	± 7	± 6	± 7	± 8	± 9	± 8	± 10	± 12	± 14	± 13	± 8	± 13	12
Alanine	274	258	277	322	352	346	409	399	419	419	419	392	374	386	342
	± 15	± 18	± 36	± 52	± 21	± 16	± 40	± 22	± 24	± 26	± 27	± 23	± 20	± 34	± 23
Taurine	96	106	98	110	115	74	96	96	160	146	106	91	107	178	165
	± 12	± 12	± 9	± 20	± 10	± 5	± 20	± 9	± 27	± 21	± 12	± 6	± 5	± 20	± 25
Glutamic acid	35	36	35	38	39	36	35	40	46	51	55	62	54	65	57
	± 5	± 3	± 4	± 5	± 3	± 4	± 4	± 5	± 4	± 4	± 7	± 5	± 6	± 5	± 10
Tryptophan	32	32	31	30	35	32	35	43	41	39	41	49	47	50	48
	± 3	± 5	± 2	± 3	± 5	± 2	± 4	± 7	± 5	± 4	± 4	± 4	± 5	± 7	± 5
Histidine	64	62	62	60	78	73	83	88	93	98	111	101	98	92	90
	± 3	± 3	± 2	± 3	± 5	± 4	± 2	± 4	± 8	± 4	± 11	± 3	± 4	± 4	± 5
Glycine	185	176	192	216	212	200	243	226	239	244	244	220	213	216	194
	± 12	± 12	± 25	± 34	± 23	± 16	± 33	± 18	± 20	± 24	± 25	± 13	± 13	± 17	± 12
Serine	92	86	84	86	105	102	108	113	125	126	131	129	120	104	110
	± 6	± 4	± 5	± 5	± 6	± 7	± 7	± 7	± 8	± 11	± 12	± 11	± 7	± 6	± 8
Tyrosine	62	56	61	64	77	65	79	77	81	89	100	99	105	110	103
	± 5	± 3	± 5	± 8	± 7	± 3	± 7	± 4	± 4	± 5	± 8	± 6	± 8	± 9	± 9
Ornithine	55	47	46	46	59	56	67	66	76	73	80	90	91	91	91
	± 5	± 3	± 3	± 2	± 5	± 3	± 5	± 4	± 5	± 3	± 5	± 4	± 2	± 2	± 4
Methionine	22	25	23	23	29	30	37	38	48	49	53	54	59	55	53
	± 3	± 2	± 1	± 2	± 3	± 1	± 3	± 5	± 3	± 2	± 3	± 2	± 4	± 3	± 4
Asparagine	45	38	36	46	46	48	57	55	61	64	66	62	57	59	52
	± 5	± 3	± 2	± 9	± 3	± 3	± 5	± 3	± 4	± 5	± 6	± 6	± 9	± 7	± 4
Phenylalanine	51	43	53	52	55	54	66	61	62	64	67	68	64	66	61
	± 3	± 2	± 6	± 6	± 10	± 2	± 7	± 2	± 2	± 2	± 4	± 3	± 2	± 2	± 4
Aspartic acid	4	5	5	6	6	4	6	5	10	8	7	8	9	13	11
	± 0	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 2	± 2	± 1	± 1	± 2	± 2	± 3

should also be considered that alanine levels were substantially elevated during AP infusion, although this amino acid is unlikely to compensate for reduced levels of some other amino acids since it is a very weak stimulus in vivo and in vitro (18,21).

Glucagon levels were higher during AP than during AS infusion. The glucagonogenic amino acids asparagine, glycine, phenylalanine, serine, and aspartate were overall imitated much better during AP infusion. Nevertheless, the contribution of circulating amino acids to glucagon release during protein-rich diets might be overestimated from the present studies since the very potent glucagonogenic amino acid glycine is extremely elevated to supraphysiological levels. Furthermore, it should be considered that AS contains more isoleucine and leucine, which have been reported to inhibit glucagon re-

lease (21). Thus, the attenuated glucagon response to AS as compared with AP might be a combined effect of reduced amounts of stimulatory and increased amounts of inhibitory amino acids. The present data suggest that circulating amino acids contribute to the postprandial rise of glucagon secretion probably between 30 and 60%.

The ingestion of the combined protein-carbohydrate meal leads to a greater insulin and an attenuated glucagon response compared with protein alone. The same shift of hormonal response can be observed when amino acids and glucose are infused together intravenously, which is in agreement with earlier reports showing that glucose augments aminogenic insulin and prevents aminogenic glucagon secretion (26,27). For glucagon levels this decrease can be observed during the first 30 min when
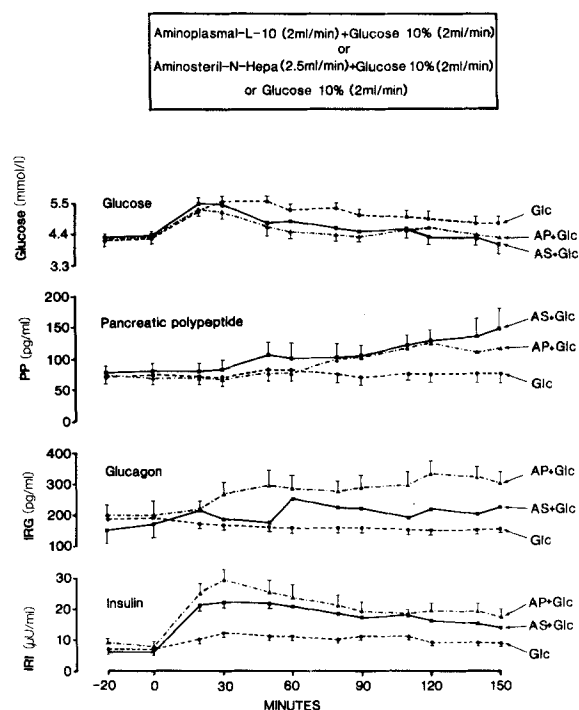
FIG. 4. Effect of the combined infusion of Aminoplasmal L-10 (AP) + glucose (Glc), Aminosteril-N-Hepa (AS) + Glc, or saline + Glc on peripheral vein plasma insulin (IRI), glucagon (IRG), pancreatic polypeptide (PP), and Glc levels (means ± SEM, n = 9).

glucose levels are still increased. Thereafter, when glucose decreases toward basal values, aminogenic stimulation of glucagon is identical.

The comparison of the effect of intravenous AP + glucose with the postprandial insulin response during the combined protein-carbohydrate meal shows that circulating substrates contribute with 60% to the total insulin response. This figure could be fairly realistic since postprandial glucose levels were matched well during the infusion and the major insulinogenic amino acids with the exception of asparagine were also in the postprandial range. Interestingly, the maximal insulin response during AS + glucose was less (43%), although a number of amino acids were substantially higher than with AP + glucose. It should be noted, though, that others were considerably lower.

The comparison of the plasma amino acid levels when the two amino acid mixtures were given with glucose with the effects when given alone demonstrates an identical pattern at least for most amino acids. In view of the increased plasma insulin levels during the combined amino acid and glucose infusion, one would have expected a reduction of those amino acids that are substantially lower in plasma

following the oral administration of carbohydrates together with protein (lysine, leucine, valine, isoleucine, proline, arginine, glutamine). However, there was no decrease when glucose was given intravenously instead of orally. The reason for this remains unclear. It can be speculated that the portal vein route of amino acid flux to the liver is of substantial importance especially when simultaneously ingested carbohydrates augment insulin secretion. In analogy to hepatic glucose uptake (28), the portal route might be of similar importance for amino acid metabolism.

Apart from circulating nutrients, intestinal hormones contribute to the stimulation of the endocrine pancreas. This has been shown for the ingestion of carbohydrate-rich meals (5–8) and it has been proposed for protein-rich meals. For the latter the assumption was based on the application of identical quantities of amino acids (9), while a comparison based on circulating amino acid levels has not been done. In spite of the still-existing problem of incomplete imitation of postprandial amino acid levels, the present data suggest that factors other than circulating amino acids contribute with 30–40% to the postprandial rise of insulin irrespective of pure protein or combined protein-carbohydrate meals. With regard to glucagon secretion, the contribution of circulating amino acids is somewhere between 30 and 60% when compared with the glucagon rise after a protein meal. When compared with the much lower rise of glucagon during a combined protein-carbohydrate meal, circulating amino acids might be responsible for 90–100% of glucagon release.

Food ingestion stimulates not only insulin and glucagon but also PP release from the islets of Langerhans (29). Previously, it has been shown by Floyd et al. (29) that circulating amino acids can contribute to the postprandial PP response following the ingestion of protein-rich meals. On the other hand, this effect was not consistent when another amino acid solution was employed (30). Our results confirm these earlier observations. With AP but not AS, there was a small but significant increase of PP levels. This suggests either that the amino acids contained in AP but not in AS, such as glutamic acid, ornithine, tyrosine, asparagine, or aspartic acid, are responsible for the difference or that some other amino acids that are more elevated in plasma during AS than AP infusion exert a very potent stimulatory or even an inhibitory effect on PP release. This would require examination of the indi-

vidual amino acids. It has been reported that arginine, leucine, and alanine do not stimulate PP levels in humans (29).

During the infusion of amino acids and glucose, PP levels rose similar to the effects observed with amino acids alone. Previously intravenous glucose has been shown either to be ineffective or to decrease basal plasma PP levels (29–34). It should be noted, though, that at the time when PP started to rise during application of amino acids + glucose (Fig. 4), glucose levels had already returned to basal values. The early rise of PP levels at 30 and 60 min as observed during AP alone was indeed abolished, which coincides with the elevation of plasma glucose levels at these time points. Insulin is probably of minor importance for this interaction since it has been shown that insulin has no effect on PP release, at least in vitro (35).

In summary, the present study demonstrates that (a) individual amino acids are increased following the ingestion of a protein or protein-carbohydrate meal; (b) the imitation of postprandial amino acid levels by infusion of available amino acid solutions for use in humans is as yet not ideal; and (c) solutions that yield fairly comparable levels of the insulinogenic amino acids (AS) or the glucagonogenic amino acids (AP) have no different effect on insulin levels, while AP gives a greater rise of glucagon and PP. In conclusion, the present data indicate the importance of the composition of amino acid solutions when the effects on the endocrine pancreas are examined with regard to the contribution of circulating amino acids to stimulation of postprandial insulin, glucagon, and PP release.

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Original Research Communications—general

Postprandial glucose and insulin responses to various snacks of equivalent carbohydrate content in normal subjects[1,2]

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ABSTRACT   To evaluate glucose and insulin responses after ingestion of snacks, we gave healthy, nondiabetic male subjects carbohydrate equivalent (25 g) snacks or isocaloric (265 kcal) snack meals in a random crossover design. Individual snacks composed of either a milk chocolate bar, granola bar, chocolate milk, peanut butter cups, yogurt, or potato chips produced similar glucose response curves. Plasma glucose concentrations were lower ($p \leq 0.05$) at 30 and 60 min postprandially than after a corresponding oral glucose challenge. In contrast, insulin responses to the snacks exhibited a two-fold variation in peak values. Isocaloric snack meals of cereal-milk, cheese sandwich-milk, and peanut butter sandwich-chocolate milk produced glucose and insulin responses similar to individual snacks. Although glucose concentrations at 60 min fell somewhat below baseline values after each snack, clinical hypoglycemia was not evident. These data clearly indicate a similarity in glycemic response among normal individuals consuming a variety of common snacks.   *Am J Clin Nutr* 1986;43:335–342.

KEY WORDS   Glucose response, insulin response, dietary carbohydrate, snacks

Introduction

Recent evidence in the literature supports the concept that glycemic responses are influenced not only by the simple sugar content of foods, but also by the type and form of complex carbohydrate (CHO) in the diet (1–8). In fact, there is so much overlap in glucose and insulin responses from simple and complex carbohydrates that foods composed of different forms of carbohydrates can no longer be considered separate entities in the diabetic exchange lists. While many of these investigations have been conducted with the goal of establishing better dietary guidelines for diabetics, others have attempted to explore mechanisms for altered metabolic responses to various foods and forms of carbohydrate in foods. These studies may ultimately change the approach toward nutrition therapy for diabetic patients (9, 10), and their outcome may

also have therapeutic utility in treating individuals with other abnormalities of lipid or carbohydrate metabolism (11).

Snacks can broadly be defined as those foods eaten between the three main meals of the day. The role of snacking has steadily gained importance in the American diet, and recent estimates report that at least 60% of the national population consumes some type of food or beverage between meals (12, 13). This has led to the concern that snacking may result in nutritionally inadequate diets because of the belief that certain snacks are junk foods and therefore contain empty calories. Because of

their relatively high simple carbohydrate content, certain types of snacks such as chocolate and confectionery products have also been accused of eliciting high insulin responses to the ingested glucose, resulting in rebound hypoglycemia followed by fatigue, hunger, and dizziness (14). However, this latter effect has never been substantiated by scientific evidence.

Very few published studies have analyzed both the glucose and insulin responses to foods or meals as consumed in their usual dietary patterns. Jenkins et al (5) have attempted to classify foods according to their glycemic index, but no estimate of insulin response was done. Since few studies evaluated common snack foods or meals as consumed by normal individuals, the present investigation was undertaken to determine the effect of ingestion of CHO equivalent snack foods and isocaloric snack meals on the glucose and insulin response in healthy, nondiabetic subjects. A wide variety of snack prototypes was chosen to investigate the glucose and insulin responses.

Methods

Subjects

Sixteen healthy, nonsmoking, and nonmedicated males with no history of diabetes participated in the study. Their mean age was 24.9 yr (range, 22–31) and their mean weight was 74.6 kg (range, 69–80). All subjects were within ±10% of the ideal body weight (according to the Metropolitan Life Insurance Tables, 1983). Eight subjects completed both Part I, a preliminary dose response study, and Part II, an evaluation of six individual snack foods/beverages; the remaining eight subjects completed Part III, an evaluation of three isocaloric snack meals. This experimental design was chosen due to the required frequency of blood sampling procedures. The study was conducted at the Milton S Hershey Medical Center/Pennsylvania State University College of Medicine, Hershey, PA, after review and approval by their Committee on Clinical Investigations. A signed, informed consent was obtained from each subject prior to the initiation of the study.

Protocol

The energy nutrient composition of each test snack is summarized in **Table 1**. Nutritional information for each snack was obtained from product labels as well as standard reference sources (15, 16). Milk chocolate bars, granola bars, chocolate milk, peanut butter cups, and milk samples were analyzed by Hershey Foods Corporation Analytical Laboratories to verify nutritional labels. Although the CHO content of each snack food (Part II) was standardized at 25 g, the total caloric value ranged from 133 to 268 kcal. The protein content ranged from 3.0 to 6.9 g, and the fat from 1.7 to 17.9 g. The snack meals (Part III) averaged

265 calories and contained 25 to 36 g CHO, 10.7 to 13.2 g protein, and 7.3 to 14.6 g fat. The total CHO content was also subdivided into two categories, simple sugars (mono- and disaccharides) and other CHO. Yogurt contained the highest percentage of simple CHO (100%) and potato chips the lowest percentage (16%). The snack meals (Part III) contained 37 to 51% of their total CHO content as simple sugar.

Test snacks were administered on a weekly basis according to a three part design. During Part I, an oral glucose tolerance test was performed at 25 and 50 g doses (weeks 1 and 2), followed by administration of a milk chocolate bar at equivalent CHO content for comparison (weeks 3 and 4). During Part II, six individual snack foods/beverages were administered in portions equivalent to 25 g CHO in a random crossover design (weeks 5–10). In Part III, an oral glucose tolerance test was performed in eight different subjects at a 25 g dose (week 11), followed by administration of three isocaloric snack meals: natural grain cereal and milk, cheese sandwich and milk, and peanut butter sandwich and chocolate milk (weeks 12–14).

The test snacks were administered at 9:00 AM on the same day each week. Subjects were permitted no food or beverage other than water after 9:00 PM on the day preceding the test. An appropriate amount of water was ingested with each test snack in order that the total meal volume was approximately 500 ml. Subjects were given 5 min to ingest the snack at which point time zero was noted. Subjects remained at rest during the test procedures and refrained from eating or drinking (other than water). Blood samples were drawn for glucose and insulin measurements in the fasting state and at 30, 60, 90, 120, 180, and 240 min postprandially.

Analytical methods

Samples for plasma glucose measurements were collected in fluoride oxalate tubes and analyzed by the hexokinase method using a FLEXIGEM™ centrifugal analyzer (Electro-Nucleonics, Inc, Fairfield, NJ). Insulin concentrations in serum were quantitated by solid-phase radioimmunoassay with a commercially available method from Diagnostic Products Corporation (Los Angeles, CA). Samples were centrifuged within one hour after collection and the plasma or serum stored at −20°C until assayed during the following week.

Data analysis

Data are presented as mean ± SEM. Area under the glucose and insulin response curves from 0 to 4 h was calculated by the linear trapezoidal method. A two-way analysis of variance with repeated measures design was used for statistical comparisons. Groups with significant F ratios were evaluated by a Newman-Keuls test.

Results

Part I

Mean plasma glucose and serum insulin responses after ingestion of 25 and 50 g CHO as either a glucose solution (92 and 185 kcal) or a milk chocolate bar (264 and 528 kcal) are

TABLE 1
Energy nutrient composition of snack foods and meals

Test snack	Serving size	Carbohydrate			Protein	Fat	Calories
		Simple	Other	Total			
		g			g	g	
Part I—Preliminary Dose Response							
Glucose solution*	2.5 fl oz (75 ml)	25.0	0	25.0	0.0	0.0	92†
Glucose solution*	5.0 fl oz (150 ml)	50.0	0	50.0	0.0	0.0	185†
Milk chocolate bar	46.3 g	23.2	1.8	25.0	3.9	15.7	264
Milk chocolate bar	92.6 g	46.4	3.6	50.0	7.9	31.7	528
Part II—Equivalent Carbohydrate Snacks							
Milk chocolate bar	46.3 g	23.2	1.8	25.0	3.9	15.7	264
Granola bar	39.0 g	12.0	13.0	25.0	3.0	10.0	200
Chocolate milk, 2% fat	207 ml	22.4	2.6	25.0	6.9	4.3	164
Peanut butter cups	49.3 g	22.8	2.2	25.0	6.5	15.2	261
Yogurt	126.2 g	25.0‡	0.0‡	25.0	5.0	1.7	133
Potato chips	50.6 g	3.9‡	21.1‡	25.0	3.6	17.9	268
Part III—Isocaloric Snack Meals							
Snack Meal 1:							
Cereal	40 g	8.4	18.8	27.2	4.0	5.6	184
Milk, 1% fat	200 ml	9.2	0.0	9.2	6.7	1.7	83
		(17.6)§	(18.8)	(36.4)	(10.7)	(7.3)	(267)
Snack Meal 2:							
Bread	28.3 g (1 slice)	1.9‡	12.1‡	14.0	2.5	1.0	75
Cheese	15 g	0.5‡	0.0‡	0.5	3.2	4.8	58
Butter	5 g	0.02‡	0.0‡	0.02	0.03	3.9	36
Milk, 1% fat	225 ml	10.3	0.0	10.3	7.5	1.9	94
		(12.72)	(12.1)	(24.82)	(13.2)	(11.6)	(263)
Snack Meal 3:							
Bread	28.3 g (1 slice)	1.9‡	12.1‡	14.0	2.5	1.0	75
Peanut butter	23 g	0.1‡	2.8‡	2.9	6.5	12.2	137
Chocolate milk, 2% fat	66 ml	7.2	0.8	8.0	2.2	1.4	52
		(9.2)	(15.7)	(24.9)	(11.2)	(14.6)	(264)

* Orange-Dex*, 100 g dextrose/10 fl oz. (Custom Laboratories, Inc, Baltimore, MD).
† USDA Agriculture Handbook No 8: 1 g glucose = 3.7 calories.
‡ Relative amount of simple and other carbohydrate estimated from Nutrients in Foods (1983).
§ Values in parentheses represent snack meal total.

summarized in **Figure 1**. Ingestion of a milk chocolate bar consistently produced a smaller (p ≤ 0.05) glucose response than an equivalent glucose solution, irrespective of CHO amount, as evidenced by the lower postprandial glucose concentrations at 30 and 60 min (Figure 1). When the CHO amount of substance was doubled from 25 to 50 g, the glycemic response was only slightly increased. These results show that at CHO levels employed in this study, glycemic response was affected more by CHO form than amount.

In contrast, insulin response at 30 min after a milk chocolate bar was similar to that of an oral glucose solution whether given as 25 or 50 g CHO doses (Figure 1). Thus, insulin re-

sponse was more sensitive to CHO amount than was glycemic response.

These observations are partially supported by the results of Peterson and Reaven (17) and Hales and Randle (18), who found that when the amount of glucose in an oral solution was doubled, the insulin response increased significantly while the glucose response was essentially unchanged.

Part II

We chose to evaluate the snack foods in Part II at CHO loads of 25 g (133–268 kcal), since the use of larger CHO doses would have led to excessive caloric intakes and portion sizes in certain snacks.
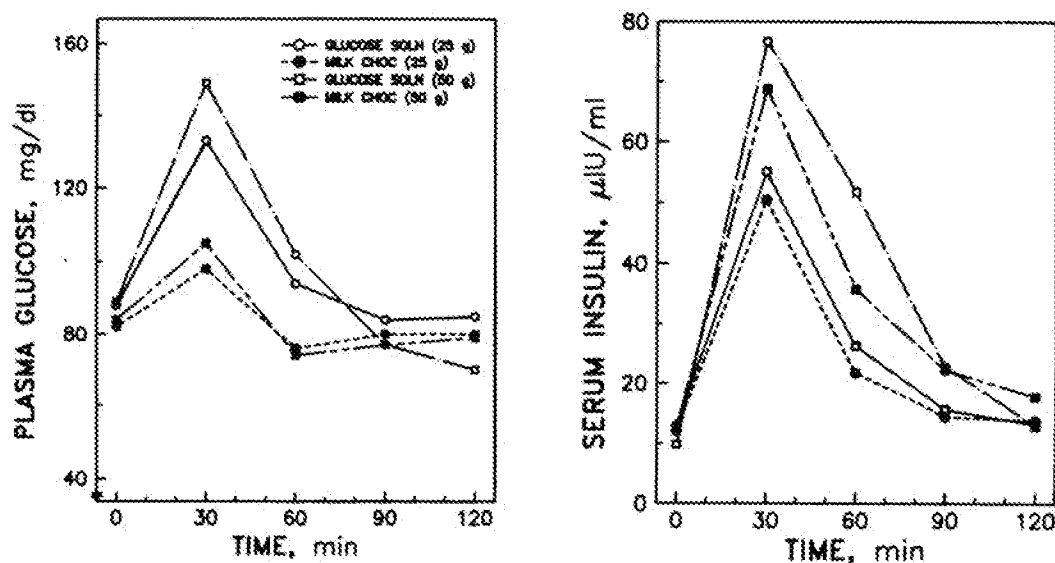
FIG 1. Mean plasma glucose and serum insulin responses after administration of a glucose solution or a milk chocolate bar at 25 and 50 g CHO equivalents (Part I).

Administration of CHO-equivalent snack foods or beverages produced similar glucose response curves, all of which were lower at 30 min than those following a glucose solution (Figure 2). Plasma glucose concentrations at 60 min fell somewhat below baseline values for all snacks except potato chips which showed an unusual response in that peak to nadir decline was prolonged compared to other snack foods. By 90 min, no differences in glucose concentrations were evident among the test snacks and glucose solution.

FIG 2. Mean plasma glucose and serum insulin responses after administration of a glucose solution or snack food at 25 g CHO equivalents (Part II).

Insulin responses to the CHO-equivalent snack foods and beverages were more variable (Figure 2). The milk chocolate bar, chocolate milk, and yogurt produced peak insulin levels similar to the glucose solution, while responses from the other snacks were lower. Potato chips produced the lowest peak insulin concentration which remained constant through 60 min, whereas insulin concentration after other snack foods decreased 51 to 74% between these times.

Area under the curve (AUC_{0-4}) calculations for snack foods showed results essentially similar to those obtained by peak glucose and insulin concentrations (**Figure 3**). The glucose AUC for each individual snack was significantly less ($p \leq 0.05$) than that of the glucose solution. The insulin AUC following the glucose solution or milk chocolate bar was significantly greater ($p \leq 0.05$) than the AUC for the granola bar, chocolate milk, peanut butter cups, yogurt, and potato chips.

Part III

The plasma glucose and insulin responses to a glucose solution and three isocaloric snack meals (263–267 kcal) are summarized in **Figure 4**. Isocaloric meals containing 25 g CHO (cheese sandwich-milk meal and peanut butter sandwich-chocolate milk meal) showed similar

glucose responses. Glucose concentrations were lower ($p \leq 0.05$) at 30 and 60 min compared to those following the glucose solution. Insulin responses for all snack meals and the glucose solution were equivalent.

An additional isocaloric meal containing a higher amount (36 g) of CHO (cereal-milk meal) produced peak glucose concentrations similar to those generated by other isocaloric meals. However, glucose concentrations decreased rapidly from 30 to 60 min after administration of the cereal-milk meal in comparison to other isocaloric meals. This effect may be due to the higher peak insulin level produced by the cereal-milk meal compared to other snack meals or glucose solution, even though the difference was not statistically significant ($p > 0.05$).

Discussion

This study evaluated glucose and insulin responses to a variety of snack foods and an equivalent oral glucose solution. Part I examined glucose and insulin responses to 25 and 50 g CHO equivalents of both an oral glucose solution and milk chocolate bar. Administration of CHO as an oral glucose solution produced higher peak glucose concentrations than when the CHO was given in a milk choc-
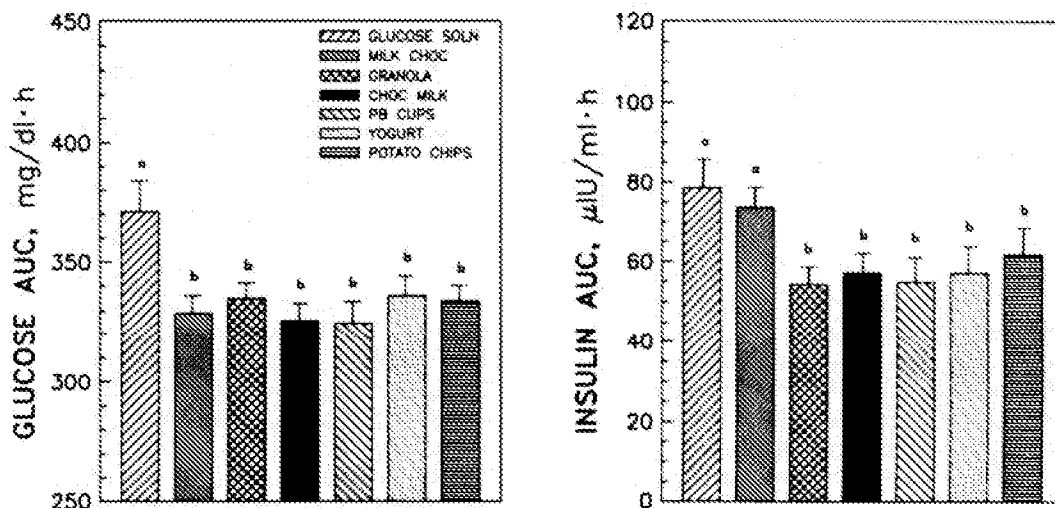
FIG 3. Mean (±SEM) areas under the curve (AUC_{0-4}) for plasma glucose and insulin response curves after administration of a glucose solution or snack food at 25 g CHO equivalents (Part II). Means with the same letter are not significantly different at $p > 0.05$ according to a two-way analysis of variance with repeated measures design.
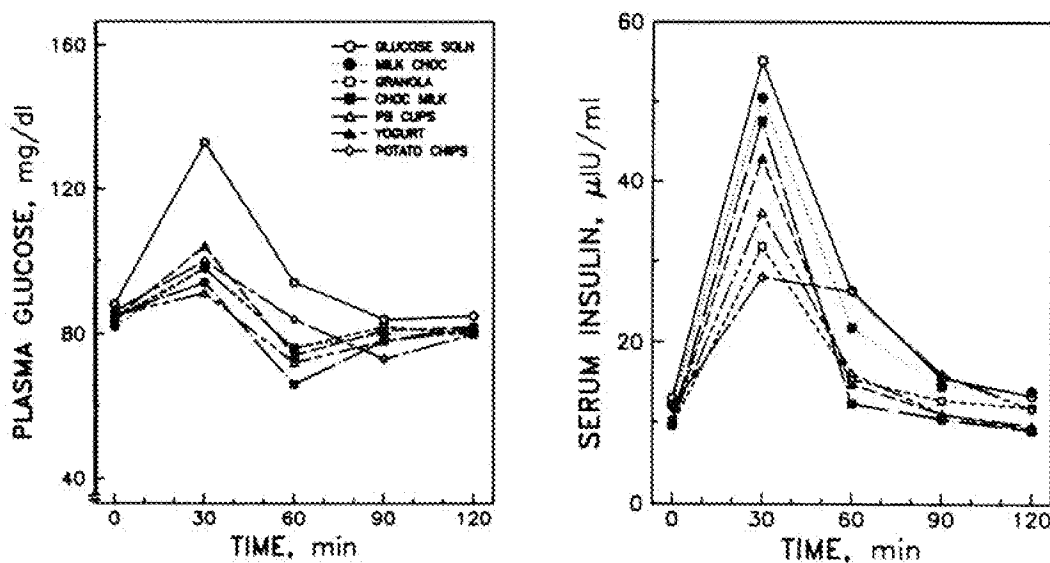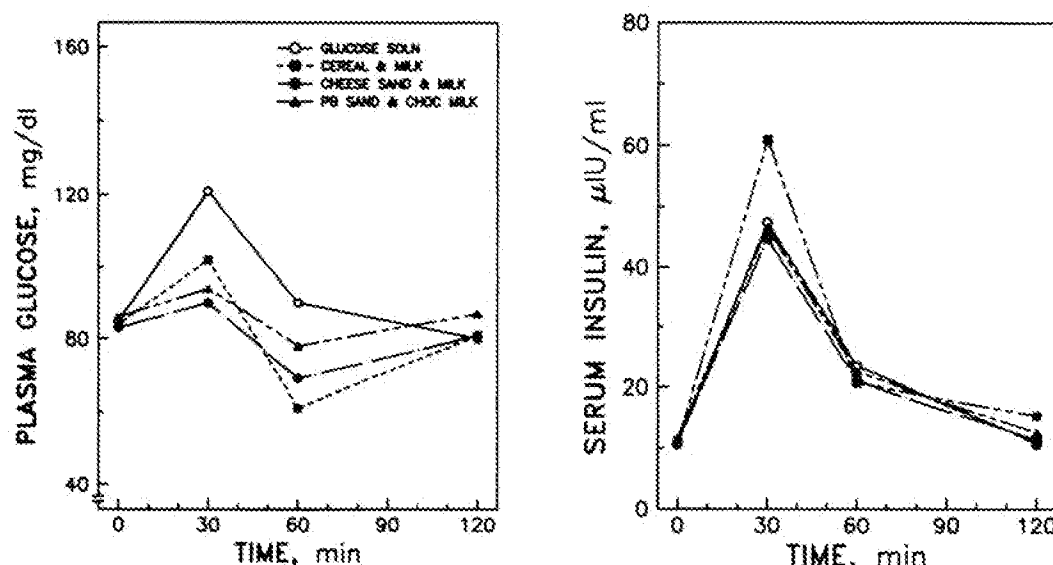
FIG 4. Mean plasma glucose and serum insulin responses after administration of a glucose solution or isocaloric snack meal (Part III).

olate bar. In contrast, peak insulin concentrations were similar after an oral glucose solution or milk chocolate bar at either 25 or 50 g CHO doses. Thus, the glycemic response of the normal male volunteers was influenced to a large extent by the particular dietary form of the CHO, whereas insulin response correlated more closely with the absolute amount of CHO. Although previous investigators also examined glucose and insulin responses to incremental amounts of an oral glucose solution in healthy (17), as well as diabetic (18) individuals, these dose–response relationships have not been systematically examined when CHO is given in dietary form. Our results using a milk chocolate bar and an oral glucose solution suggest that while similar relative dose–response relationships exist, *absolute* response is dependent on dietary form of CHO.

In Part II six typical snack foods (milk chocolate bar, granola bar, chocolate milk, peanut butter cups, yogurt, and potato chips) were administered at 25 g CHO loads, since this amount gave caloric intakes and portions typical of snacks normally consumed in the American diet. Although the energy nutrient composition and the ratio of simple to complex CHO content of the snacks were different (Table 1), similar glucose response curves were

obtained in the subjects. These responses were less than those elicited by corresponding amounts of an oral glucose solution (Figure 2). Since insulin responses to the snacks were more variable, and in some cases (milk chocolate bar, chocolate milk, and yogurt) did not differ from the oral glucose solution, factors other than CHO amount influence insulin release.

Alterations in glycemic responses to foods may be due to changes in *1*) CHO dosage and form, *2*) gastric emptying time, *3*) gastrointestinal and pancreatic hormone secretion by coingested protein and fat, and *4*) bioavailability or digestibility of the CHO source. In addition, recent studies found that insulin can be released during the preabsorptive or cephalic phase of digestion simply by the sweet taste of the food (19). Glucose response can also be diminished by coingested fat (20, 21) and dietary fiber (22). These effects, probably secondary to delayed gastric emptying, may partially explain the reduced glycemic responses observed in this study after snack food administration.

Previous investigators (1–8) analyzed glycemic responses to a wide variety of single foods or mixed meals, but none have dealt with typical snack foods commonly ingested

at lower CHO amounts. The majority of these studies also focused on diabetic patients with the ultimate goal of improving their nutrition therapy. Jenkins (5) developed the concept of calculating a glycemic index for each food (AUC_{0-2} test food/AUC_{0-2} glucose solution) so that foods with low glycemic indices could be identified for therapeutic use. Glycemic indices were not calculated in the present study since these measurements have been standardized to administration of 50 g CHO loads (5). The reason for this standardization is that administration of 25 g CHO may artificially raise the glycemic index when compared to 50 g CHO (5).

With the increasing role of snacking in the American diet, it is important to evaluate glucose and insulin responses to different types of snacks in order to provide an objective basis for proper selection of snacks to fulfill energy and nutrition requirements. Traditionally, it has been assumed that a milk chocolate bar would produce a glucose response equivalent to an oral glucose solution as illustrated in recent studies (23) that recommended substitution of D-glucose by a milk chocolate bar to treat diabetic hypoglycemia. However, our studies do not support this hypothesis. Other commonly-made assumptions that the rise in glucose following ingestion of a chocolate bar produces rebound hypoglycemia are also not supported. While glucose concentrations at 60 min fell somewhat below baseline values after the snacks (including milk chocolate equivalent to 25 or 50 g CHO, ie one or two 1.45 oz Hershey bars, respectively), clinical hypoglycemia was not evident.

Similar plasma glucose concentrations were present after ingestion of various isocaloric snack meals (Part III). These curves were similar to those obtained after the individual snack foods in Part II indicating that variation in the ratio and type of simple and complex CHO did not alter glycemic responses. Insulin responses to isocaloric test meals were also similar, confirming previous observations that insulin release is equivalent after isocaloric meals (24). However, it was observed that snack foods which were isocaloric (potato chips, peanut butter cups, and milk chocolate, Table 1) produced differences in insulin response (Figure 2). These contrasting responses, which

may be due to the vastly different composition of the snack foods, suggest that the caloric content of a food or meal is not the primary regulator of insulin release.

In summary, this study defined and compared the effects of various typical snacks on glucose and insulin response in healthy adults. Glucose response was similar among snacks, but lower than that following an equivalent glucose solution. A more variable insulin response occurred after the foods, substantiating that factors other than absolute CHO amount influence insulin release. These results indicate the importance of insulin sensitivity to different foods in the maintenance of normal glucose response. ▣

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Effect of protein dose on serum glucose and insulin response to sugars[1-3]

Gene A Spiller, PhD; Christopher D Jensen, RD; Thomas S Pattison, MS; Carol S Chuck, RD; James H Whittam, PhD; and James Scala, PhD

ABSTRACT To clarify the effects of protein on insulin and glucose response to sugars, 14 healthy normal-weight males and females were fed test meals containing 0, 15.8, 25.1, 33.6, and 49.9 g protein along with ~58 g carbohydrate. Serum samples were obtained at fasting time zero and 15, 30, 60, and 120 min postprandial. Mean areas of the glucose curves above fasting decreased with increasing protein dose. Protein-containing meals produced significantly lower ($p < 0.01$) areas than the protein-free meal and the relationship between blood glucose area and protein dose was significant ($p < 0.001$). Protein-containing meals produced significantly greater ($p < 0.01$) insulin areas compared with the protein-free meal. However, no differences in insulin areas among the protein-containing meals were observed. These data support previous studies showing a blood glucose moderating and insulin-enhancing effect of protein ingestion. *Am J Clin Nutr* 1987;46:474-80.

KEY WORDS Protein, carbohydrate, glucose response, insulin response, glycemic index, diabetes

Introduction

Protein ingestion and oral or intravenous administration of amino acids have been shown to stimulate insulin secretion and moderate postprandial hyperglycemia in normal and type II diabetic subjects (1-5). It is possible also that the beneficial effect that legumes have had in terms of their glycemic index (6) and long-term dietary treatment in diabetes (7) may relate to their higher-protein content by comparison with other starchy foods, in addition to their dietary fiber, type of starch, and content of other components.

To date, the few studies that have examined the serum insulin and glucose response to various protein doses have yielded mixed results. Day et al (8) fed a constant carbohydrate load from whole foods while varying the protein dose (3.6-75 g protein) and detected differences in glucose responses at 60 and 90 min between the high- and low-dose protein meals as well as differences in insulin levels at 60 min between the high-dose protein meal and the remaining meals. More recently, Nuttall (9) reported a protein-dose effect on serum insulin response when 0-50 g protein was fed with a 50 g glucose solution. However, no differences have been seen in the responses at the intermediate-protein doses. Jenkins et al (6) were unable to detect a significant change in the plasma glucose area above baseline in diabetic subjects fed a carbohydrate meal

of wholemeal bread and cottage cheese vs wholemeal bread alone where the protein difference was only 12 g.

The dose-response study reported here was undertaken to define the protein dose at which a significant effect on serum insulin and glucose response might be expected in healthy subjects.

Subjects and methods

Subjects

Healthy individuals, eight females and six males aged 28-59 y (mean age 38 y), who were employees of the research center and free of diabetes or family history of diabetes participated after voluntary informed consent was obtained. Subjects had previously participated in similarly designed pilot studies that had examined the effects of various liquid test meals on glucose and insulin response to simple sugars. Consequently, subjects were familiar with the experimental regimen. Before participation in these studies, all subjects were given a standard oral glu-

TABLE 1
Test meals*

Ingredients	Pro0	Pro1	Pro2	Pro3	Pro4
g					
Maltodextrin	23.9	23.2	23.2	23.7	23.2
Fructose	18.3	19.0	19.1	19.0	19.1
Lactose	15.4	15.2	15.7	15.9	15.9
Total carbohydrate (g)	57.6	57.4	58.0	58.6	58.2
Milk proteins	—	5.2	8.1	10.8	16.0
Soy proteins	—	10.6	17.0	22.8	33.9
Total protein (g)	0.0	15.8	25.1	33.6	49.9

* Ingredients for test meals came from the following sources: maltodextrin—Maltrin M100, Grain Processing Corporation, Muscatine, IA; fructose—D-Fructose 54016, Roche Chemical Division, Nutley, NJ; lactose—Edible Lactose, Land O'Lakes, Minneapolis, MN; milk proteins—Meloskim 500 (nonfat dry milk), Dairyland Products, Inc, Savage, MN, and TMP 1220 (total milk proteinate), New Zealand Milk Products, Inc, Petaluma, CA; soy proteins—Pro-Fam G-902 (soy protein isolate), Grain Processing Corporation, Muscatine, IA.

cose tolerance test and found to be normal in this regard. Their mean percent of desirable body weight was 104 ± 8% (range: 91–126%) using the 1959 Metropolitan Life Insurance Co tables for persons of medium frame. The design and procedures followed in the study were in accord with the Helsinki Declaration as updated in Tokyo, Japan in 1975.

Design

Subjects adhered to an alcohol-free, high-carbohydrate diet (> 200 g/d) for 72 h before each test-meal feeding and were monitored by dietary records. Exercise was discontinued 24 h before and fasting was instituted 12 h before each test meal was administered. On the mornings of the test meals, venous blood was drawn and then meals (**Table 1**) were consumed over a 2 min interval. Additional venous blood samples were drawn at 15, 30, 60, and 120 min after test meals were ingested. No exercise and additional food were allowed during the experimental period. The test meals were administered at least 6 d apart and on a given day subjects received the same test meal in the following order: 0 g, 25.1 g, 49.9 g, 15.8 g, and 33.6 g protein. The mean fasting blood insulin levels were similar on each test meal day. Mean fasting blood glucose levels were similar on the days the protein-containing meals were fed but lower ($p < 0.01$) on
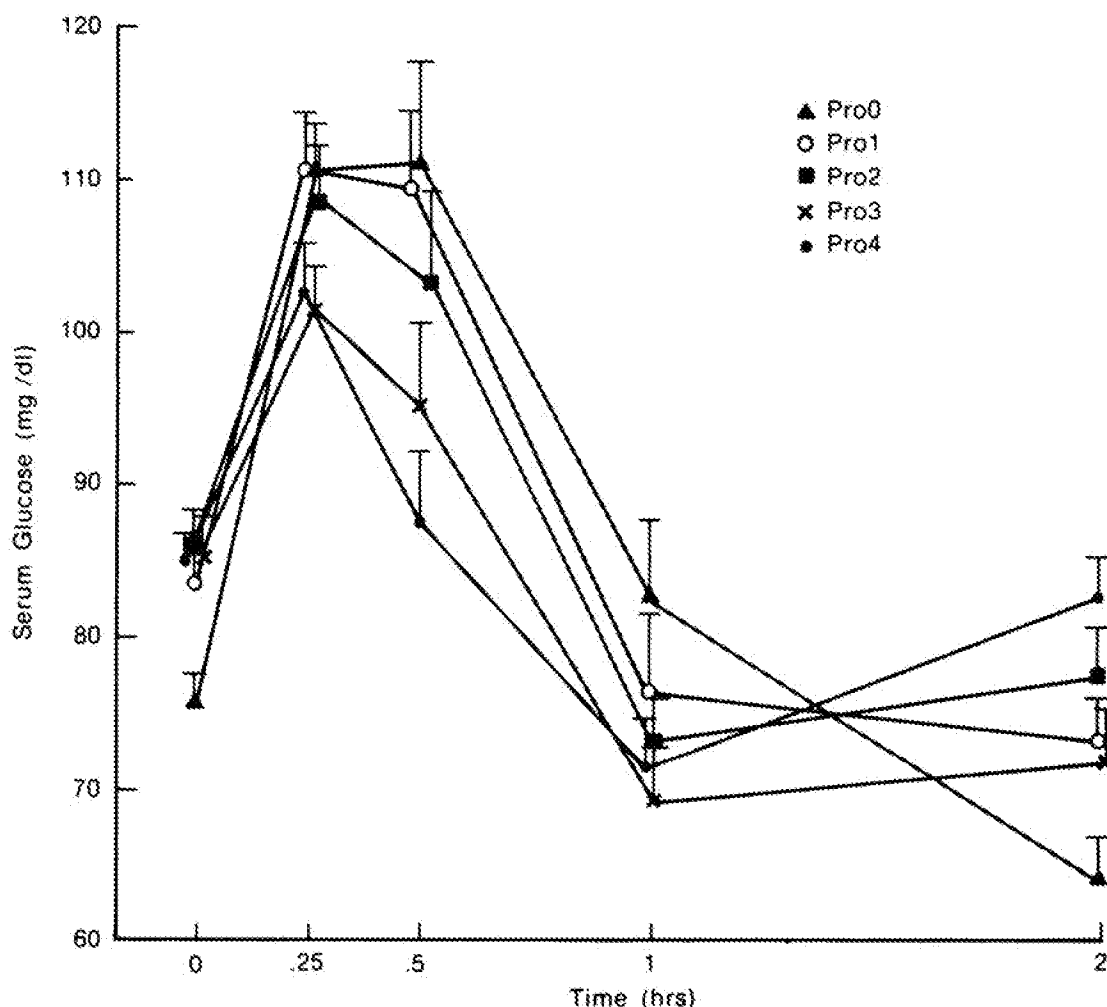
FIG 1. Serum glucose response to protein dose. Data are means ± SEM.

the day of the protein-free test meal (Fig 1). One male subject was unavailable for the 25.1 g protein test meal and one female was unavailable for the 33.6 g protein test meal. Otherwise, all subjects ingested all test meals.

Test meals

Test meals (Table 1) were dissolved in 375 mL cold tap water. Each meal provided ~58 g carbohydrate, various amounts of protein, and trace amounts of fat. The levels of protein, fat, and carbohydrate of the various raw materials in the test meals were obtained from manufacturer specifications and confirmed by the standard methods of the Association of Official Analytical Chemists (10) for protein (#2.057), fat (#16.064), and fructose and lactose (#31.138). The degree of polymerization (DP) of the maltodextrin was characterized by high-pressure liquid chromatography (HPLC) (11) using a APX-42A column (Bio-Rad Laboratories, Richmond, CA). The approximate average glucose polymer molecular weight was 1800 and the average number of anhydrous glucose units per molecule was 11.1. The DP was distributed as follows: 0.5% DP1, 2.7% DP2, 4.3% DP3, 3.7% DP4, 3.1% DP5, 5.7% DP6, 7.1% DP7, 4.5% DP8, 3.1% DP9, 1.6% DP10, and 63.7% DP10 and above (Grain Processing Corporation, Muscatine, IA, personal communication).

Blood glucose and insulin analyses

Serum glucose was obtained by an automated glucose oxidase method (SMA24, Technicon, Terrytown, NJ) and serum insulin

was measured using a radioimmunoassay kit (Phadeseph Insulin RIA, Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistical analyses

A one-way analysis of variance was used to test for significant differences in fasting serum glucose and insulin levels between the test meals and responses to the test meals. Differences were calculated from the actual values in the case of fasting levels and as increments from fasting values or incremental areas above fasting for the responses to the test meals. Incremental areas were calculated by computing and summing the individual areas described by the trapezoids above the fasting values between time zero and each separate sampling time. When the F statistic indicated significance, the method of least significant differences was used to compare means.

Results

Compliance

Diet records indicated that subjects complied with the high-carbohydrate, alcohol-free regimen before the feeding of test meals.

Glucose response

The apparent maximum mean serum glucose rise from fasting for each protein-containing formula was seen at

FIG 2. Areas above baseline under the glucose curves. Data are means ± SEM. Areas are significantly different ($p < 0.01$) if they do not share a common superscript letter.

15 min postconsumption, whereas, the test meal without protein (Pro0) appeared to reach its maximum increment at the 30 min mark (Fig 1). Each of the protein-containing test meals produced lesser maximum mean glucose increments as compared with the Pro0 test meal. Expressed as a percentage of the protein-free test meal, these differences were significant ($p < 0.01$) except for Pro1 (15.8 g protein). In addition, Pro3 and Pro4 (33.6 g and 49.9 g protein, respectively) yielded significantly smaller ($p < 0.01$) maximum glucose increments than did Pro1 and Pro2 (15.8 g and 25.1 g protein, respectively). The time estimated for the return to baseline of the mean glucose levels was found to decrease as the protein dose increased (Fig 1). The estimated mean times were 35, 43, 45, 58, and 76 min for Pro4, Pro3, Pro2, Pro1, and Pro0, respectively. The time differences between test meals were statistically significant ($p < 0.01$) except for Pro3 and Pro2 ($p > 0.05$). The maximum fall from baseline in mean serum glucose levels detected within 2 h after consumption did not differ significantly for any of the test meals ($p > 0.05$).

The mean areas of the glucose curves above baseline for each test meal are shown in **Figure 2**. The glucose areas produced by ingestion of the protein-containing test meals were significantly lower ($p < 0.01$) than the area corresponding to Pro0. In addition, the mean areas for the protein-containing test meals decreased with increasing intakes of protein. The differences in areas between Pro3 and Pro4 versus Pro1 were significant ($p < 0.01$). When the logarithm of these areas was plotted vs protein dose, a straight line resulted, suggesting a first-order relationship between moderation of the hyperglycemic response and dose of protein (**Fig 3**). The correlation coefficient was 0.986 ($p < 0.001$).

Insulin response

For all of the test meals, the apparent maximum mean serum insulin increments from fasting were seen at 30 min (**Fig 4**). Pro0, the test meal containing the sugars alone, produced the lowest maximum rise from fasting, although none of the differences were significant ($p > 0.05$). The only pattern related to protein dose was the insulin increment above baseline observed at the 2-h mark. The increment was progressively larger with increasing protein dose. The mean serum insulin increment above baseline for Pro4 was significantly larger ($p < 0.01$)
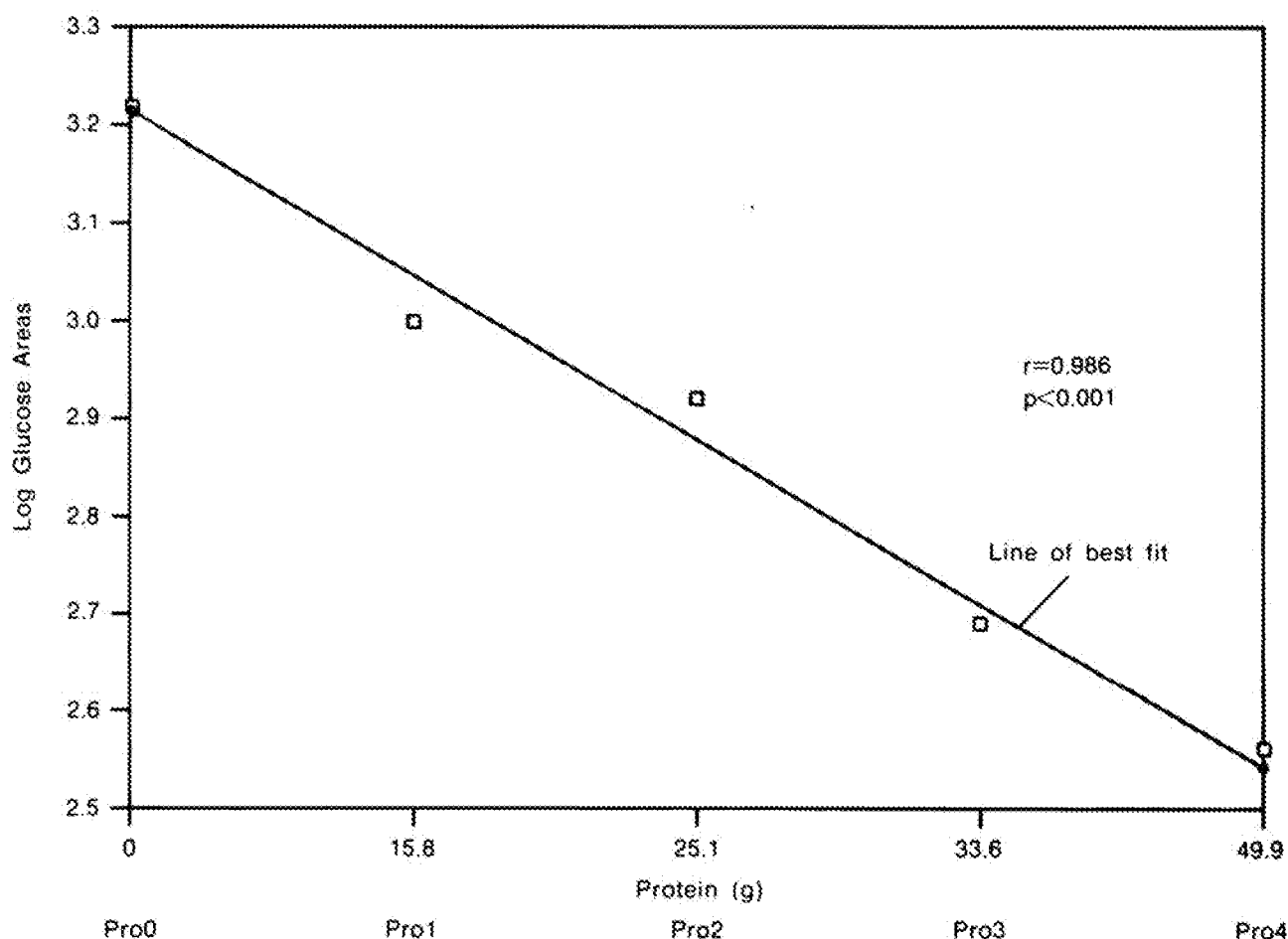
FIG 3. Logarithms of the areas above baseline under the glucose curves versus protein dose.
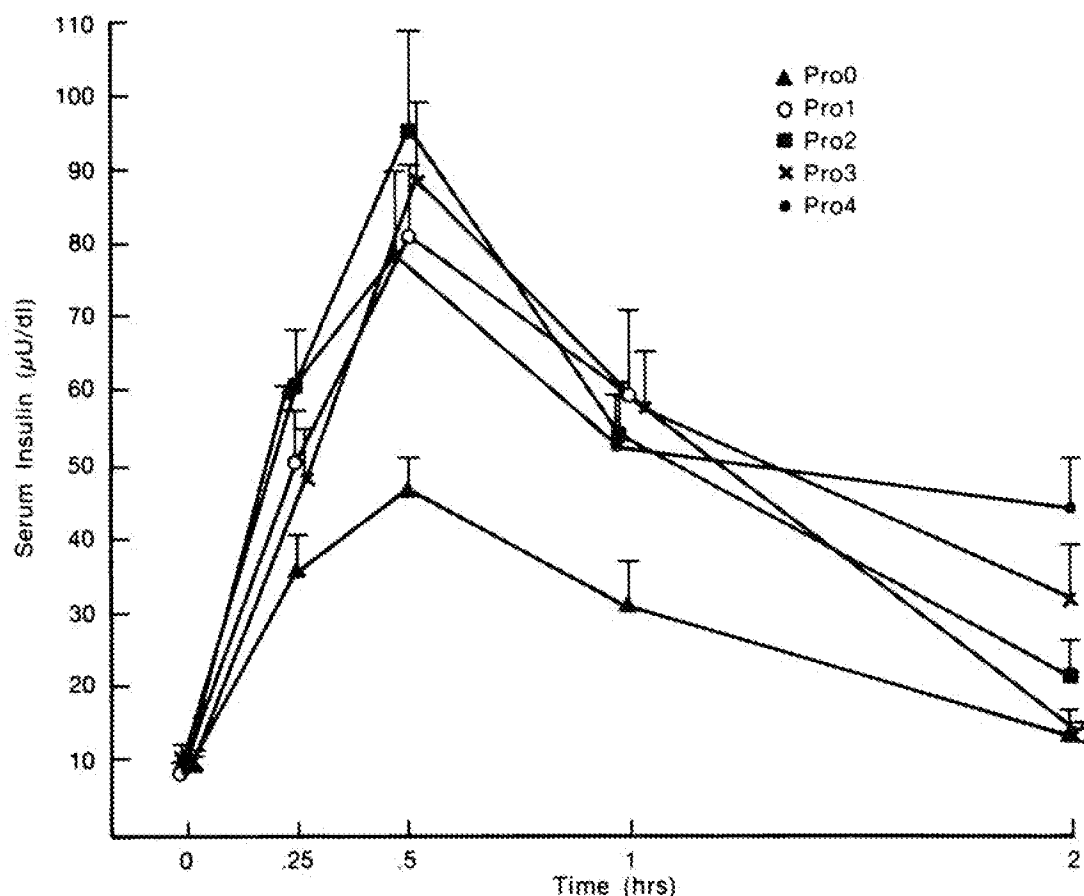
FIG 4. Serum insulin response to protein dose. Data are means ± SEM.

than the increment produced by Pro3, which in turn was larger ($p < 0.01$) than the responses to Pro1 and Pro0 (Fig 4). Mean insulin levels did not return to baseline within 2 h for any of the test meals.

The mean areas under the serum insulin curves above baseline for each of the protein-containing test meals were significantly greater ($p < 0.01$) than the area corresponding to Pro0 (Fig 5). However, none of the differences in areas between protein doses achieved statistical significance at $p < 0.05$ within the 2-h sampling period.

Discussion

The lower maximum mean serum glucose increments and earlier times at which these increments were seen, along with the shorter periods of time required for glucose levels to return to baseline and the reduced areas under the glucose curves, confirm the results of previous studies in both normal (3) and type II diabetic (12) subjects. These studies found that after a carbohydrate load glucose elevations are clearly affected by concomitant protein ingestion if substantial differences exist in the protein levels in the meals. The finding that serum insulin response is enhanced by adding protein to a carbohydrate load is also in agreement with these studies. In addition, our results

can explain the failure of investigators in some situations to see an effect of protein. Jenkins et al (6) reported in diabetic subjects that a meal of wholemeal bread and cottage cheese (22.1 g protein, 50 g carbohydrate) did not yield a significantly lower plasma glucose area above baseline than did a meal of wholemeal bread alone (12.1 g protein, 50 g carbohydrate). A comparison of the effects of Pro1 (15.8 g protein) and Pro2 (25.1 g protein) in our study showed similar responses. However, when the protein doses were increased further, significant reductions in area did occur. In fact, a clear dose response effect was demonstrated from zero to ~50 g protein.

Areas under the serum insulin curves did not support a protein dose-response effect. However, mean insulin levels did not return to baseline within 2 h (Fig 4). The fact that the insulin increment above baseline at the 2 h mark was increased with increasing protein dose suggests that, had the blood sampling period been extended, significant differences might have been observed.

Day et al (8) fed 3.6–75 g protein with a standard amount of carbohydrate from whole-food sources but detected little variation in the effects of protein dose on glucose and insulin response in normal subjects over 90 min. However, interpretation of their results is made difficult by the fact that meals were fed at noon and the fat content,
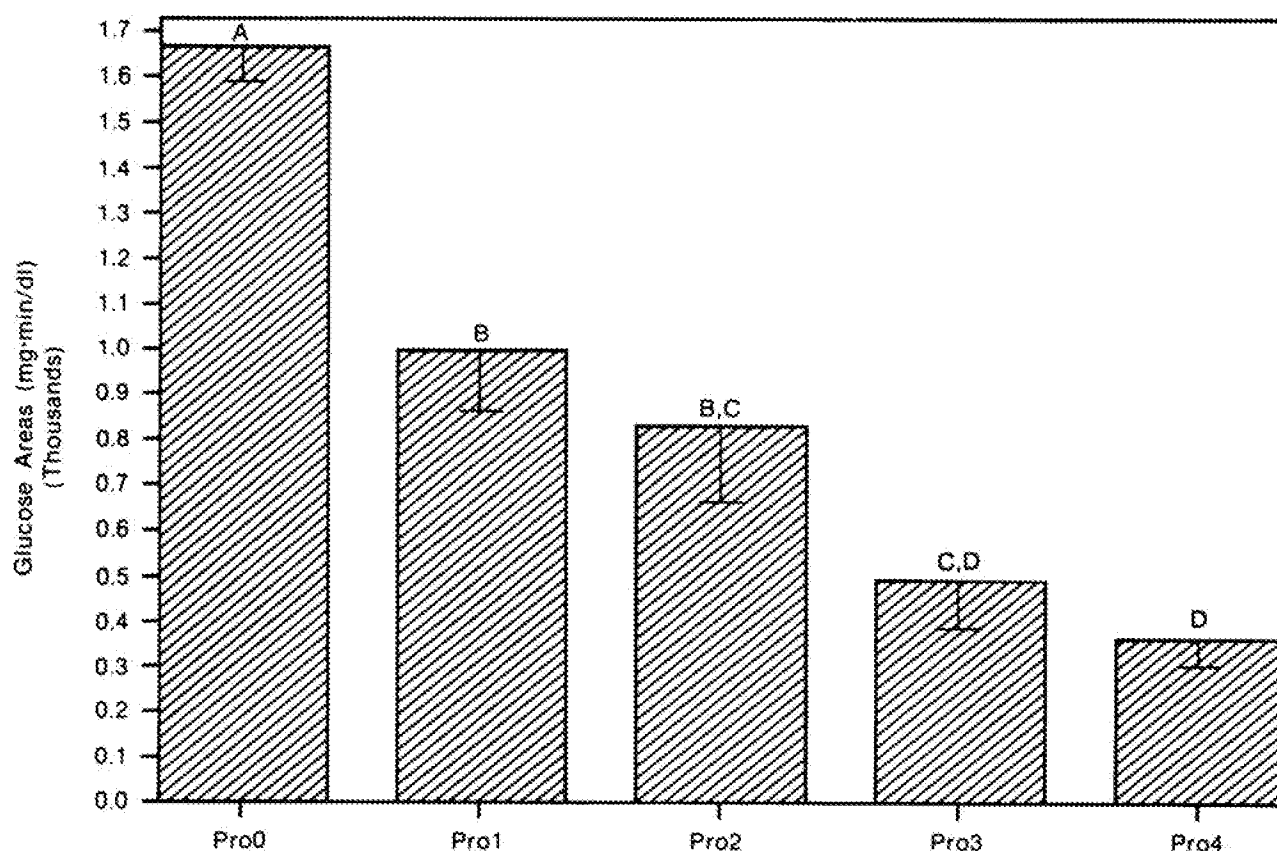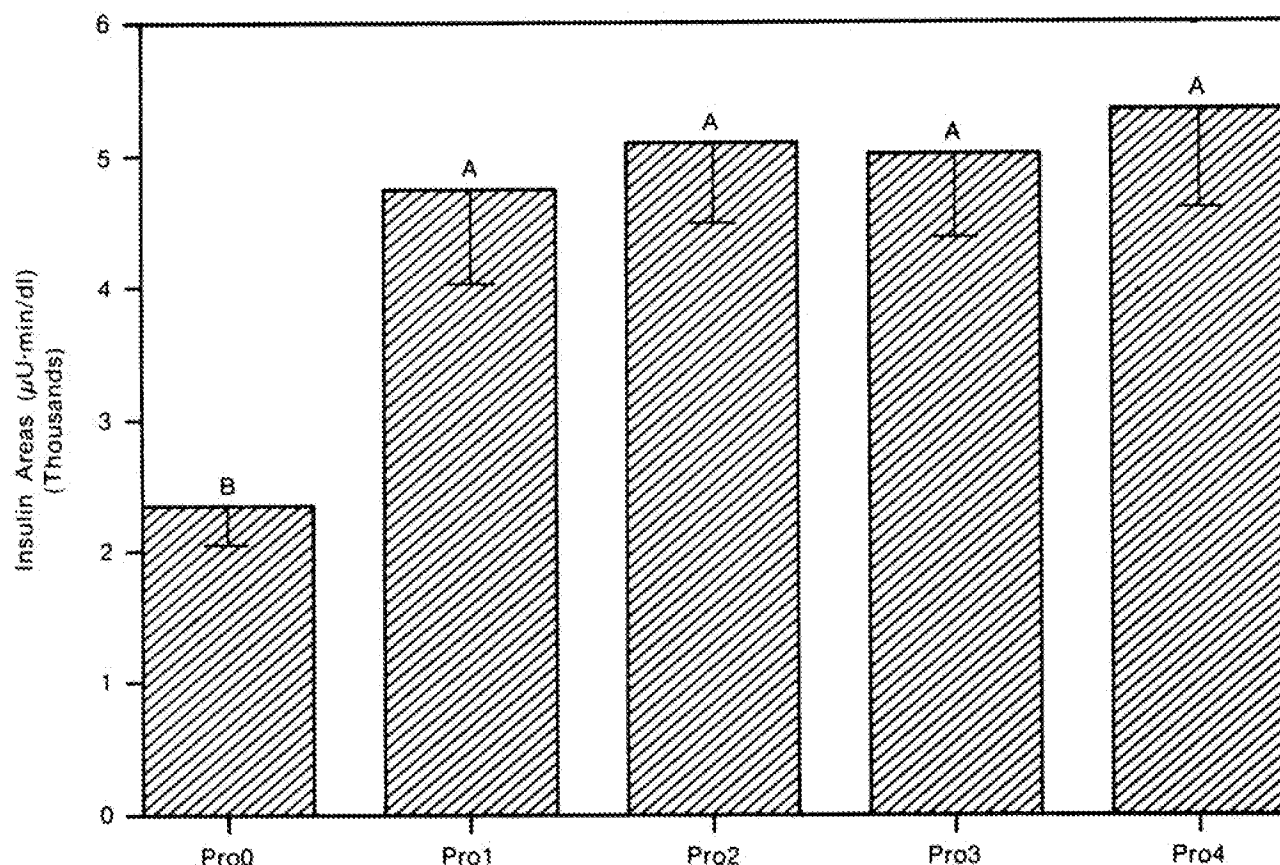
FIG 5. Areas above baseline under the insulin curves. Data are means ± SEM. Areas are significantly different ($p < 0.01$) if they do not share a common superscript letter.

known to alter gastric emptying time (13), varied between diets. The test meals used in our study were designed to contain negligible amounts of fat to avoid this difficulty.

Nuttall et al (9) recently fed 0, 10, 30, and 50 g protein with 50 g glucose to five mild untreated type II diabetic subjects whose percent of ideal body weight was 123 ± 23%. Only the 50 g protein treatment yielded a significantly lower net area (net area equal to area below baseline subtracted from area above baseline) under the glucose curve as compared with the glucose treatment alone. Insulin areas were significantly greater for both the 30 and 50 g protein treatments as compared with the glucose treatment alone or in conjunction with 10 g protein. Fajans et al (14) have suggested that obesity in type II diabetics may lead to an exaggerated insulin response to protein dose. However, comparison of the insulin and glucose responses as indicated by areas above baseline or net areas (data not shown) under the respective curves from our mostly normal-weight healthy subjects to that of Nuttall's mildly overweight type II diabetic subjects suggests that the diabetic subjects were less sensitive to the lower doses of protein.

In summary, protein appears to exert a clear dose effect on glucose response as determined by mean areas above baseline under the glucose curve in normal fasting subjects

fed test meals consisting primarily of simple sugars and oligosaccharides. Due to the length of the blood sampling time in this study, similar conclusions regarding effects of protein dose on insulin cannot be made. Comparison of these results with those of mildly overweight type II diabetics fed similar protein levels point to a greater sensitivity to protein ingestion on the part of normal-weight healthy subjects. These findings are contrary to previous reports of greater insulin response observed with obese diabetics and indicate the need for additional research to clarify the effects of protein ingestion on serum insulin and glucose response in individuals with differing glucose tolerance status.

We are not suggesting at this point that the protein intake of the diabetic diet should be increased. Our results indicate that meal protein intakes must demonstrate differences of between ~10–20 g, when given in a liquid meal form, before significant differences in glucose response are observed in healthy subjects ingesting mostly simple sugars and oligosaccharides. Further studies comparing liquid test meals with traditional foods are needed before the relevance of these findings to the clinical setting is clear. It also should be noted that the carbohydrate distribution of these test meals is not typical of suggested diabetic meal plans. Advice to increase protein intake in

the diabetic diet is premature at a time when there is concern over high levels of dietary protein and renal damage (15, 16).

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Dietary Carbohydrates: Effects on Self-selection, Plasma Glucose and Insulin, and Brain Indoleaminergic Systems in Rat

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The aim of the study was to investigate the effect of different dietary carbohydrates such as corn starch, sucrose, fructose and glucose on carbohydrate and protein self-selection and on arterial and venous concentrations of glucose and insulin, and brain indoleamines in rats. Fructose and sucrose feeding induced the lowest food intakes which were due respectively to a lower carbohydrate and protein selection. The present data showed that feeding with dietary glucose as the main carbohydrate source gave the highest glycemic response, the lowest one being found with fructose and corn starch, and an intermediate one with sucrose feeding. The insulin response to the dietary carbohydrates followed a somewhat different pattern with the highest insulin secretion observed after fructose feeding whereas highly variable and inconsistent results were obtained following corn starch, sucrose and glucose feeding. Feeding chemically different sugars was also characterized by decreased serotonin synthesis in the raphe nuclei, brainstem and thalamus, and increased 5-HT synthesis in the hypothalamus of rats fed fructose when compared to glucose fed animals. The present results highlight the importance of considering the nature of dietary carbohydrates in the regulation of feeding.

INTRODUCTION

Pharmacological and nutritional studies support brain serotoninergic mediation of the proportions of protein and carbohydrate selected from a dietary choice (see review by Blundell, 1988). Peripheral control of food intake and food preference has also been postulated by the existence of glucoreceptors in the liver sending signals to the central nervous system by way of the vagi (Russek, 1963; Niijima, 1983; Tordoff and Friedman, 1986). Moreover, the role of absorbed nutrients in feeding behaviour has been demonstrated by studies showing that glucose and free fatty acids can directly modulate the firing of glucose-sensitive neurons in the hypothalamus (Oomura, 1976). In the context of most of the experiments conducted with dietary choices, carbohydrates referred either to corn starch or to other simple sugars such as sucrose or maltodextrins. Carbohydrates, however, are a complex group of nutrient substances showing marked differences in the way they are handled and used by the

TABLE 1

Composition of diets (dry weight g/100 g diet)[a]

	0%	60%
Casein	0·00	60·00
Corn oil	10·00	10·00
Carbohydrate[b]	78·65	18·65
Alphacel	5·00	5·00
Mineral mix	5·25	5·25
Vitamin mix	1·10	1·10
Energy density (kcal/g)	4·05	4·05

[a] Prepared in our laboratory.
[b] 0% diet: corn starch, sucrose, fructose or glucose; 60%: corn starch.

body. Some carbohydrate food, being more or less insulinogenic and glycemic, are likely to provoke quite different effects on the brain than others, which in turn may affect ingestive behaviour. The aim of the present study was therefore to investigate the effect of different dietary carbohydrates such as corn starch, sucrose, fructose and glucose, on carbohydrate and protein self-selection and on arterial and venous concentrations of glucose and insulin in the rat. In addition, tryptophan, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were assayed in the hypothalamus, thalamus, raphe nuclei and brainstem in order to obtain a further understanding of the serotoninergic systems affected.

METHODS

Animals

Male Sprague–Dawley rats (Charles River Laboratories, Québec) weighing 338 ± 3 g were housed in individual cages in a room maintained at $23 \pm 2°C$ and 55% humidity. A reversed 12 h-light–12 h-dark cycle set with lights on from 1800 to 0600 hrs was used to facilitate the execution of the protocol. The experiment was conducted during the spring. After 7 days of adaptation to environmental conditions, rats were randomly divided into eight experimental groups of 18 animals each, according to their body weight. Rats were given *ad libitum* water and a choice of two isocaloric diets (0 and 60% casein) (Table 1) for 8 h each day (0600–1400 hrs; dark cycle) using corn starch, sucrose, fructose or glucose as the main carbohydrate source for each two experimental groups, during 16 consecutive days. Two identical spill-proof food containers for choice-regimen were rotated daily at random from side to side and among all animals tested.

At the end of the 16-day experimental period, food was removed after the 8-h feeding period as usual and one of each of the four groups of animals fed a different carbohydrate source was laparatomized for arterial and venous blood samplings and, simultaneously, the four other groups were guillotined for brain extraction. Briefly, animals were anesthetized with halothane, laparatomized and, within 2 min, arterial (abdominal aorta) and venous (hepatic portal vein) blood samples were

taken using EDTA-coated monovettes syringes (Sarstedt). Blood was kept on ice and immediately centrifuged at 2500 rpm for 15 min in a refrigerated (4°C) Sorvall RC-5B and the plasma was then stored at −80°C until assayed. Groups of animals were guillotined without anesthesia and brains were set aside within 45 seconds and hypothalamus, thalamus, raphe nuclei and brainstem were dissected out on an ice-cold plate, immediately frozen on dry ice and stored at −80°C until analysed.

Biochemical Determinations

Plasma glucose content was determined by the spectrophotometric method of Fisher Scientific Limited (Diagnostic Division, D.K. 1200-31/33/36), and insulin by a radioimmunoassay procedure of Bio-Endo Inc. (RIA Kit, KT-1001).

Brain tissues were weighed frozen and placed in 500 µl ice cold 0.1 M $HClO_4$ containing 40 ng isoproterenol as internal standard. Tissues were thoroughly homogenized using a motor driven glass--teflon tissue grinder and centrifuged at 30 000 g for 30 min at 4°C. The resultant supernatant was decanted into a clean polypropylene test tube and frozen once prior to analysis by HPLC with electrochemical detection in order to precipitate perchlorate salts which otherwise remain in solution. Samples were held in the dark and injected (5 µl) in a refrigerated WISP 712 autosampler (Waters, Montréal, Canada) maintained at 4°C. Chromatographic separations were performed at 30°C (Waters column heater) on an Ultrasphere I.P. C-18 analytical column (250 × 4·6 mm I.D., 5-µm spherical particles) (Beckman, Canada). This column was protected by a 0·5 µm SSI pre-column filter and a Brownlee C-18 guard column (15 × 3·2 mm I.D., 7·2-µm spherical particles) (Chromatographic Specialties, Brockville, Ontario, Canada). The mobile phase comprised 10 mM monobasic sodium phosphate, 5 mM dibasic sodium phosphate, 0·35 mM sodium 1-octanesulfonic acid (SOSA), 5 mM EDTA, 10% methanol, 2% acetonitrile and 0·25% tetrahydrofuran (THF). Prior to the addition of the organic modifiers, the aqueous phase was filtered through a 0·22 µm Millipore type GS filter (Toronto, Canada). The final apparent pH was adjusted to 4·25 with concentrated phosphoric acid. The mobile phase was continually recycled and degassed with a stream of helium and delivered at 1·0 ml/min using an SSI Model 222 pump (SSI, Pennsylvania, U.S.A.) with an auto-backwashing pump head and a coil-type pulse dampener (Waters). Detection was achieved using an EG & G model 400 thin-layer amperometric detector (EG & G, Princeton, U.S.A.). The glassy carbon electrode had an applied potential of 740 mV vs. an Ag/AGCl reference electrode. Signals from the detector were integrated by peak height using a Spectra-Physics Computing Integrator SP-4290 (Technical Marketing Associates, Montréal, Canada).

Chemicals

L-tryptophan, 5-HT (creatinine sulfate complex isoproterenol-HCl) and 5-HIAA (dicycloexylammonium salt) were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Phosphoric acid and HPLC-grade methanol, acetonitrile and THF were obtained from Coledon Laboratories (Ontario, Canada). SOSA was purchased from Regis Chemical Company (Morton Grove, IL, U.S.A.). All other chemicals were Fisher reagent grade (Fisher, Montréal, Canada). Casein, corn oil, corn starch, sucrose, fructose, glucose, alphacel and mineral and vitamin mix were supplied by ICN Nutritional Biochemicals (Montréal, Canada).

TABLE 2

Effect of dietary carbohydrate source on food intake and selection in rats[a]

Dietary carbohydrate sources	Total food intake (g)	Carbohydrate intake[b] (g)	Protein intake[c] (g)	Carbohydrate energy[d] (%E-CHO)	Protein energy[e] (%E-PRO)
Corn starch	$22 \cdot 6 \pm 0 \cdot 2^B$	$12 \cdot 9 \pm 0 \cdot 1^A$	$4 \cdot 9 \pm 0 \cdot 1^C$	$56 \cdot 6 \pm 0 \cdot 4^A$	$21 \cdot 0 \pm 0 \cdot 4^C$
Sucrose	$20 \cdot 0 \pm 0 \cdot 2^C$	$10 \cdot 6 \pm 0 \cdot 1^B$	$5 \cdot 1 \pm 0 \cdot 1^C$	$52 \cdot 6 \pm 0 \cdot 3^B$	$25 \cdot 0 \pm 0 \cdot 3^B$
Fructose	$18 \cdot 4 \pm 0 \cdot 2^D$	$6 \cdot 7 \pm 0 \cdot 1^C$	$7 \cdot 8 \pm 0 \cdot 1^B$	$35 \cdot 4 \pm 0 \cdot 4^D$	$42 \cdot 3 \pm 0 \cdot 4^A$
Glucose	$28 \cdot 9 \pm 0 \cdot 4^A$	$12 \cdot 7 \pm 0 \cdot 2^A$	$10 \cdot 2 \pm 0 \cdot 2^A$	$43 \cdot 2 \pm 0 \cdot 5^C$	$34 \cdot 4 \pm 0 \cdot 5^B$
F value and probabilities (*DF* 3, 2186)	364·1(0·0001)	451·8(0·0001)	478·5(0·0001)	640·6(0·0001)	641·0(0·0001)

[a] Effects of four types of carbohydrate (corn starch, sucrose, fructose and glucose) on food selection from 0 and 60% protein diets were tested repeatedly for 8-h daily periods. Results (means ± *SEM*) are derived from the total number of observations from day 3 to 16. There were 36 animals in each of the corn starch, sucrose, fructose and glucose fed groups. Values followed by the same letter are not significantly different.

[b] Carbohydrate intake (g) = [intake from 0% diet (g) × 0·7865] + [intake from 60% diet (g) × 0·1865]. Since fat, fibre, vitamin and mineral content is fixed at 0·2135 g/g of diet, carbohydrate content is the only variable in, and the major constituent of the non-protein intake.

[c] Protein intake (g) = [intake from 60% diet (g) × 0·60].

[d] Carbohydrate energy (%E-CHO) = [carbohydrate intake (g) × 4 (kcal/g carbohydrate)] ÷ [total food intake (g) × 4·05 (kcal/g)] × 100.

[e] Protein energy (%E-PRO) = [protein intake (g) × 4 (kcal/g protein)] ÷ [total food intake (g) × 4·05 (kcal/g)] × 100.

Statistical Analysis

Mean, standard error of the mean (*SEM*), Student's *t*-test and a one-way analysis of variance (ANOVA) followed by a Duncan's new multiple range test were used (SAS Institute, 1985; Steel and Torrie, 1980).

RESULTS

Food Intake and Selection and Body Weight

Table 2 presents results of food intake and selection for the different dietary carbohydrate sources. For all groups of animals, the selection patterns stabilized within 2 days. However, mean food intake and selection varied widely among the different groups. Groups of animals self-selecting glucose and fructose showed the highest and the lowest total food intakes, respectively, paralleled by the highest protein intake with glucose as dietary carbohydrate source and by the lowest carbohydrate intake with fructose. The percentages of total caloric intake ingested as protein (%E-PRO) and carbohydrate (%E-CHO) were significantly different among all groups of animals. Indeed, animals fed fructose selected the highest %E-PRO, followed by the glucose, sucrose and corn starch groups. The opposite situation was observed with regards to the %E-CHO, the latter being the highest in the corn starch fed animals, followed by the sucrose, glucose and fructose fed groups. During the experimental period, the highest body weight gain was observed in animals fed glucose (4·92 ± 0·21 g/day) and sucrose (4·24 ± 0·34) whereas the lowest body weight

TABLE 3

Effect of dietary carbohydrate source on arterial and venous glucose and insulin in rats self-selecting diets[a]

Dietary carbohydrate sources	Arterial	Venous
Glucose (mmol/l)		
Corn starch	10.3 ± 0.4^C	8.1 ± 0.3^B
Sucrose	13.4 ± 0.6^B	10.8 ± 0.6^A
Fructose	11.1 ± 0.7^C	8.8 ± 0.4^B
Glucose	15.4 ± 0.4^A	12.0 ± 0.2^A
F value and probabilities (DF: 3, 67)	17.6(0.0001)	17.0(0.0001)
Insulin (μU/ml)		
Corn starch	29.9 ± 2.1^C	29.5 ± 1.5^B
Sucrose	55.9 ± 3.4^A	64.9 ± 5.1^A
Fructose	30.7 ± 2.9^C	38.5 ± 3.0^B
Glucose	40.8 ± 4.5^B	37.4 ± 3.0^B
F value and probabilities (DF: 3, 67)	11.8(0.0001)	20.4(0.0001)

[a] Arterial and venous glucose and insulin levels were determined at the end of the 16-day experimental period.

Results are expressed as the mean $\pm SEM$.

Values followed by the same letter are not significantly different.

gains were found in fructose- (3.61 ± 0.19) and corn-starch- (2.92 ± 0.44) fed groups, the latter being significantly lower ($p<0.01$) than those of glucose- and sucrose-fed animals, $F(3,148) = 7.49$, $p<0.0001$.

Blood Parameters

Analysis of arterial and venous glucose and insulin concentrations (Table 3) revealed significant differences between groups of animals. In the groups fed corn starch and fructose, the absolute arterial and venous glucose levels were significantly lower than those found in rats fed sucrose and glucose. Among experimental groups of animals, those fed glucose had the highest arterial glucose concentrations, and both glucose- and sucrose-fed animals had the highest venous glucose levels. Although not statistically significant, arterio-venous (AV) differences of blood glucose were 44% higher in the glucose fed group (3.4 ± 0.4) than in the other groups (corn starch: 2.2 ± 0.4; sucrose: 2.6 ± 0.3; fructose: 2.3 ± 0.4), $F(3,67) = 2.03$, $p<0.118$. The most elevated arterial and venous insulin concentrations were found in the sucrose-fed group. Whereas the fructose group showed a consistent and elevated AV difference of insulin (-7.8 ± 1.3), suggesting an enhanced insulin secretion, the values obtained for AV differences of insulin in the corn starch (0.4 ± 2.3), sucrose (-9.0 ± 4.2) and glucose (3.4 ± 4.87) groups were inconsistent, $F(3,67) = 3.58$, $p<0.0188$.

Brain Parameters

Changes in brain tryptophan, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content are presented in Table 4. In most brain areas examined, ptophan

TABLE 4

Effect of dietary carbohydrate source on brain tryptophan, 5-HT and 5-HIAA content in rats self-selecting diets[a]

Brain structures	Dietary carbohydrate sources				F value and probabilities DF: 3, 67
	Corn starch	Sucrose	Fructose	Glucose	
Tryptophan					
Hypothalamus	4019 ± 174^C	9234 ± 917^{AB}	10882 ± 1184^A	7493 ± 441^B	16·05(0·0001)
Thalamus	3886 ± 149^C	8937 ± 704^A	10194 ± 472^A	5255 ± 444^B	41·64(0·0001)
Raphe nuclei	3181 ± 124^C	5246 ± 680^B	15384 ± 861^A	4150 ± 99^{BC}	106·42(0·0001)
Brain stem	3286 ± 133^B	9253 ± 723^A	8435 ± 206^A	3959 ± 176^B	62·21(0·0001)
5-HT					
Hypothalamus	459 ± 31^D	1249 ± 54^A	1130 ± 17^B	568 ± 36^C	131·21(0·0001)
Thalamus	395 ± 12^A	$68·9 \pm 3·52^B$	$68·4 \pm 3·53^B$	433 ± 28^A	161·55(0·0001)
Raphe nuclei	512 ± 17^B	$99·3 \pm 6·24^C$	$91·7 \pm 7·03^C$	620 ± 14^A	358·84(0·0001)
Brain stem	371 ± 14^B	$66·0 \pm 3·93^C$	$91·12 \pm 6·23^C$	$403 \pm 9·2^A$	300·12(0·0001)
5-HIAA					
Hypothalamus	549 ± 25^C	1005 ± 44^{AB}	960 ± 34^B	1082 ± 25^A	57·54(0·0001)
Thalamus	614 ± 19^{AB}	563 ± 37^{BC}	494 ± 18^C	649 ± 22^A	8·16(0·0001)
Raphe nuclei	833 ± 49^A	490 ± 17^C	580 ± 23^B	889 ± 22^A	37·71(0·0001)
Brain stem	446 ± 18^A	$264 \pm 9·7^C$	333 ± 12^B	417 ± 12^A	37·77(0·0001)
5-HIAA/5-HT					
Hypothalamus	$1·27 \pm 0·09^B$	$0·83 \pm 0·06^C$	$0·85 \pm 0·03^C$	$2·04 \pm 0·15^A$	37·41(0·0001)
Thalamus	$1·56 \pm 0·04^C$	$8·39 \pm 0·52^A$	$7·47 \pm 0·36^B$	$1·61 \pm 0·12^C$	142·09(0·0001)
Raphe nuclei	$1·62 \pm 0·60^C$	$5·35 \pm 0·48^B$	$6·70 \pm 0·37^A$	$1·45 \pm 0·05^C$	78·68(0·0001)
Brain stem	$1·21 \pm 0·03^B$	$4·19 \pm 0·24^A$	$3·98 \pm 0·33^A$	$1·04 \pm 0·04^B$	66·76(0·0001)

[a] Brain tryptophan, 5-HT and 5-HIAA content were determined at the end of the 16-day experimental period.

Results are expressed in nanograms per gram of tissue (mean $\pm$ SEM).

Values followed by the same letter are not significantly different.

concentration was significantly higher in the sucrose- and fructose-fed groups than in the corn-starch- and glucose-fed animals. Sucrose and fructose feeding also induced more elevated 5-HT levels in the hypothalamus but tremendously lowered 5-HT levels in the extrahypothalamic brain areas when compared to corn-starch and glucose feeding. The changes in 5-HIAA levels showed a similar profile as those found for 5-HT, but the differences between the values obtained with sucrose and fructose feeding and those with corn-starch and glucose feeding were less marked than those found for 5-HT. The groups of animals fed sucrose and fructose displayed significantly higher 5-HIAA to 5-HT ratios in the thalamus, raphe nuclei and brain stem and a significantly lower 5-HIAA to 5-HT ratio in the hypothalamus than the two other groups. The effects of fructose feeding on the serotoninergic parameters can be differentiated among all groups on the basis of the fructose-induced highest tryptophan content and 5-HIAA to 5-HT ratio in the raphe nuclei. On the other hand, the highest hypothalamic 5-HIAA to 5-HT ratio was found in the glucose-fed animals. Glucose and corn starch feeding elicited similar effects on the serotoninergic parameters measured, with the exception of glucose-induced higher hypothalamic tryptophan, 5-HT and 5-HIAA concentrations and 5-HIAA to 5-HT ratio, and

higher 5-HT levels in raphe nuclei and brain stem in comparison with the corn starch-induced changes.

Discussion

The present results have shown that different dietary carbohydrate sources produce differential food intake and selection of carbohydrate and protein, therefore affecting not only peripheral glucose and insulin availability, but also causing different disposition in brain tryptophan, 5-HT and 5-HIAA content in selective sites.

Palatability of food, reflecting positive and negative hedonic effects, has been proposed as one of the decisive factors underlying food intake (Young, 1967; Booth et al., 1986). The relative sweetness of the carbohydrates offered in the present study did not, however, seem to correlate with food intakes and selection, since corn starch, a carbohydrate which is relatively tasteless, and sucrose, which is sweet, induced similar total food intakes and proportions of carbohydrate and protein selected. In rats given a choice between glucose and fructose solutions, food preference did not depend on the taste of the sugar solutions; rats drinking the same volume of each (Tordoff et al., 1990). Arimanana and Leathwood (1984) have also demonstrated that independently of organoleptic properties such as odour, taste and texture, adult rats respond quickly to the protein/carbohydrate composition of the food they eat. This would indicate that the determinant of a choice is likely to be of metabolic origin.

In the present experimental conditions, glucose and sucrose feeding produced different patterns of selection, characterized by a sucrose-related lower total food intake mainly due to a lower protein intake, as compared to glucose. Fructose feeding was also associated with a lower total food intake, due to a decrease in both carbohydrate and protein intakes when compared to those intakes obtained with glucose feeding. In contrast with the present results, Hill et al. (1980) reported the work of Castonguay, who offered groups of rats varying concentrations of either glucose or sucrose, concurrently with Purina Chow, and observed that all groups maintained equal levels of caloric intake and the same percentage of the intake as protein. The effects of fructose on the liver must be considered in the regulation of food intake (Rowland and Stricker, 1978). Liver is sensitive to its own metabolism (possibly glycogen content) and relays satiety signals to the brain via the vagus nerve to inhibit the central control of meal initiation (Stricker and Rowland, 1978; Friedman and Granneman, 1983). In rats, 75% of oral fructose is converted to both liver and muscle glycogen (Niewoehner et al., 1984) and such increased glycogen storage would be expected to result in depressed caloric consumption.

Although their total food intake and pattern of selection did not differ significantly, the sucrose group gained weight at a significantly greater rate than the corn starch group, in agreement with the work of Hill et al. (1980). On the other hand, the significant differences in total food intake and pattern of selection with sucrose and glucose feeding were associated with similar growth rates. These data suggest that different carbohydrate sources bring different metabolic consequences.

The present results indicate that dietary glucose, considered as the most insulinogenic sugar (Reiser and Hallfrisch, 1987), caused significant increase in total food intake mainly due to increase in protein intake, relative to the other carbohydrate sources. Glucose AV difference was also higher with glucose feeding, suggesting an

enhanced glucose utilization. This is in part consistent with the metabolic effects of sugars and their implications in food intake in which an enhancement of appetite has been explained by the insulinogenic response that provokes a decrease in plasma glucose, an increase in glucose utilization, and an eventual deficit of available glucose (Larue-Achagiotis and Le Magnen, 1985; Campfield *et al.*, 1985). However, the present data do not demonstrate the involvement of insulin in food intake of glucose-fed animals. Among the literature supporting a role of insulin in food intake a single composite diet was generally used (see review from Woods *et al.*, 1986). The self-selection paradigm in the present work has shown that animals which were fed glucose selected more protein thereby increasing their total food intake. A different outcome for a balanced diet versus a high-protein one with regards to insulin secretion is legitimate.

The present data also show that feeding with dietary glucose as the main carbohydrate source gave the highest glycemic response in both arterial and venous blood, the lowest one with fructose and corn starch, and an intermediate one with sucrose feeding. These results are consistent with those obtained by Jenkins *et al.* (1981) using acute test meals, feeding 50 g of either glucose, sucrose or fructose to normal volunteers. Rodin *et al.* (1988) have also reported that, in humans, fructose is associated with markedly lower postabsorptive plasma glucose levels when compared to glucose.

Glucose is the primary substrate for the central nervous system (CNS) metabolism. When this substrate is insufficient for the metabolic needs of CNS neurons, compensatory behavioral and autonomic responses are elicited to elevate circulating glucose levels and increase its availability (Himsworth, 1970; Smith and Epstein, 1969). Since fructose is a metabolic fuel readily utilized by the liver (Reiser and Hallfrisch, 1987), and therefore does not easily cross the blood–brain barrier (Rapoport, 1976), the higher percentage of energy selected as protein (furnishing substrate for gluconeogenesis) and the high insulin secretion observed in response to fructose feeding in the present study, could be part of such a compensatory response. The present experiment shows that the absolute plasma glucose values did not differ reliably as a function of the differences in carbohydrate intakes but rather according to the type of sugar presented. Moreover lower arterial and venous glucose levels were associated with corn starch and fructose feeding and higher levels with sucrose and glucose feeding. The insulin response to the different carbohydrates followed a somewhat different pattern and the AV differences of insulin suggested the highest insulin secretion after fructose feeding whereas highly variable and inconsistent results were obtained for AV difference of insulin following corn starch, sucrose and glucose feeding. The differential selection of protein and carbohydrate could possibly explain the different insulin selection patterns, since in addition to stimulating glucose utilization insulin also has pronounced effects on amino acid and lipid metabolism as well as a number of other intracellular processes. There are then a variety of ways in which insulin secretion can be stimulated and fructose feeding may have caused insulin release because of the associated high percentage of energy selected as protein and its amino acid content. Fructose, a monosaccharide, is a component of the disaccharide sucrose, and even if insulin is not required for its absorption (see review by Van den Berghe, 1978), some studies have reported that fructose is insulinogenic when blood glucose is elevated to postprandial levels (Dunnigan and Ford, 1975; Reiser *et al.*, 1987). This may not have the same regulatory effect on food intake. Indeed, in the present work both fructose and sucrose feeding were associated with

lower total food intake but were opposed in terms of carbohydrate (high with sucrose but low with fructose) and protein (high with fructose but low with sucrose) selections. Evidence from many studies support a potential role of plasma-derived insulin in the regulation of food intake. It has been demonstrated that raising plasma insulin levels by feeding, glucose infusion, or insulin infusion results in significant elevation of insulin levels in cerebrospinal fluid of rats, and this would, in turn, suppress food intake (Figlewicz et al., 1987). In the present study, the elevated insulin secretion without causing severe hypoglycemia in the fructose fed group, would be consistent with the suppressed eating effect observed in this group. Such a fact has been proposed by Vander Weele et al. (1980), who showed that during infusion of small amounts of insulin subcutaneously to rats via osmotic minipumps, the smallest doses of insulin had no effect at all, whereas, slightly larger doses, which raised plasma insulin without causing hypoglycemia, reduced food intake and body weight, and larger doses of insulin, which caused hypoglycemia, increased food intake.

It has been shown that manipulation of 5-HT metabolism produces marked changes in food intake, food preference and body weight, with the most consistent effect being the suppression of food intake by experimental treatments that directly or indirectly activate 5-HT receptors (see Blundell, 1988 for review). Under carefully chosen experimental conditions to ensure reliable changes in brain 5-HT metabolism during the period that food selection is measured in adult male rats (using 0 and 55% casein diets), Arimanana et al. (1984) obtained results consistent with the prediction that accelerating brain 5-HT synthesis should lead to a preference for protein and lowering brain 5-HT should diminish protein preference. The present results show that, in all brain areas of the fructose-fed group, the concentrations of tryptophan, 5-HT and the value of the 5-HIAA to 5-HT ratio varied in the opposite direction to those observed in glucose-fed animals. This is of particular interest in view of the fact that fructose and glucose groups also displayed opposite feeding behaviour. Despite higher concentrations of tryptophan, the lower concentrations of 5-HT and 5-HIAA in the thalamus, raphe nuclei and brain stem of rats fed fructose may reflect decreased 5-HT metabolism, even though the ratio of 5-HIAA to 5-HT in these structures was significantly higher when compared to that of rats fed glucose. On the other hand, the latter brain parameters were suggestive of a higher 5-HT metabolism in the hypothalamus of the fructose-fed group. Thus it can be suggested that the fructose-feeding associated lower total energy intake coupled with a high %E-PRO and a low %E-CHO is reflected by low serotoninergic activity in extrahypothalamic areas and high serotoninergic activity in the hypothalamus, whereas the selection patterns and serotoninergic events with glucose feeding were in the opposite direction. Ashley et al. (1979) have demonstrated that long-term depletion of brain 5-HT induced by PCPA, 5, 7-dihydroxytryptamine or lesions of the raphe nuclei results in chronic suppression of protein intake, enhancement of carbohydrate intake but unaltered total food energy consumed. On the other hand, if synaptic levels of 5-HT are increased by injecting small doses of fenfluramine (a 5-HT reuptake blocker) to rats given access to diets containing 5 or 45% casein during an 8-h dark period, there is a transient rise in the proportions of calories eaten as protein, consistent with the known action of fenfluramine which causes an initial release of 5-HT into the synaptic cleft and hence increases activity in sero-oninergic neurons (Fuxe et al., 1975), and this pattern of intake was followed by a reduction in total energy intake (Wurtman and Wurtman, 1977). However, when considered over the full day, fenfluramine reduces the absolute intake of protein,

but not of carbohydrate (Li and Anderson, 1983), which is consistent with the long-term effect of depleting neurons of 5-HT and eventual decreased activity of 5-HT in the neurons (Garattini et al., 1975). Leathwood and Ashley (1983) reported that in adult rats offered pairs of diets containing either 0 or 60% casein, there was a highly significant inverse correlation between energy intake and whole brain 5-HT, 5-HIAA or 5-HT + 5-HIAA levels and this was independent of the protein or carbohydrate intake. In these previous studies, however, assessment of 5-HT in the whole brain did not allow a precise anatomical location of the effects. The present data obtained with sucrose and fructose feeding tend to partly support the latter findings since both groups showed the lowest total food intakes but opposite carbohydrate and protein selection patterns and resulted in hypothalamic parameters suggesting a higher 5-HT synthesis and extra-hypothalamic ones in the opposite direction. However, fructose feeding was associated with a significantly higher %E-PRO and a significantly lower %E-CHO than those of the other experimental groups; a higher tryptophan and lower 5-HT and 5-HIAA levels in the raphe nuclei than those of the corn-starch- and glucose-fed animals were also found. The cell bodies of serotonin-containing neurons in the mammalian central nervous system are localized primarily in the raphe nuclei of the brainstem (Steinbusch, 1984), which have been selectively affected by fructose feeding in the present work. The hypothalamus, however, comprises different nuclei with different roles in feeding behaviour which could have been differentially affected by the chemically different carbohydrates, but the present study did not allow such anatomical localization. In a recent trial adult male rats were infused bilaterally in the VMH with fructose (200 nmol/h) for 7 days and offered macronutrients in three separate diet rations. A 50% reduction in energy intake and carbohydrate selection was observed during fructose infusion when compared to the pre- and postinfusion periods (L. Thibault and K. Nagai, unpublished results).

In the rat, the first relay nucleus for visceral and taste information is the nucleus of the solitary tract. After a major projection to the parabrachial nucleus in the pons, the pathway bifurcates with one branch going to the ventro-basal complex of the thalamus and then to the gustatory cortex whereas the other branch goes to the hypothalamus and limbic system (Norgren and Leonard, 1973; Ricardo and Koh, 1978). The present results suggest that inputs from feeding with chemically different sugars are integrated at various levels in the brain, characterized by lowered 5-HT synthesis in raphe nuclei, brainstem and thalamus but enhanced synthesis in the hypothalamus with fructose and sucrose feeding when compared to glucose feeding, thus supporting the hypothesis of a brain hierarchical organization of feeding behaviour, as also suggested by Norgren et al. (1977).

In conclusion, the present results suggest that chemically different carbohydrates alter total food intake and selection in a two choice system (0 and 60% casein diets), and are reflected by different arterial and venous glucose and insulin levels as well as by distinct brain serotoninergic metabolism responses in different neuroanatomical systems. The brain serotonin systems were affected by the pattern of carbohydrate and protein selection but similar selection patterns elicited by two different carbohydrate sources could also result in different brain serotoninergic metabolism, thus showing the importance of considering the nature of dietary carbohydrates in feeding regulation. Although the present work seems to relate peripheral and central events to the selection pattern and total food intake elicited by different dietary car-

bohydrates, the possibility remains that the carbohydrate source *per se* is the responsible factor.

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Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate[1-3]

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ABSTRACT

Background: Protein induces an increase in insulin concentrations when ingested in combination with carbohydrate. Increases in plasma insulin concentrations have been observed after the infusion of free amino acids. However, the insulinotropic properties of different amino acids or protein (hydrolysates) when co-ingested with carbohydrate have not been investigated.
Objective: The aim of this study was to define an amino acid and protein (hydrolysate) mixture with a maximal insulinotropic effect when co-ingested with carbohydrate.
Design: Eight healthy, nonobese male subjects visited our laboratory, after an overnight fast, on 10 occasions on which different beverage compositions were tested for 2 h. During those trials the subjects ingested $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ carbohydrate and $0.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of an amino acid and protein (hydrolysate) mixture.
Results: A strong initial increase in plasma glucose and insulin concentrations was observed in all trials, after which large differences in insulin response between drinks became apparent. After we expressed the insulin response as area under the curve during the second hour, ingestion of the drinks containing free leucine, phenylalanine, and arginine and the drinks with free leucine, phenylalanine, and wheat protein hydrolysate were followed by the largest insulin response (101% and 103% greater, respectively, than with the carbohydrate-only drink; $P < 0.05$).
Conclusions: Insulin responses are positively correlated with plasma leucine, phenylalanine, and tyrosine concentrations. A mixture of wheat protein hydrolysate, free leucine, phenylalanine, and carbohydrate can be applied as a nutritional supplement to strongly elevate insulin concentrations.   *Am J Clin Nutr* 2000;72:96-105.

KEY WORDS   Insulin secretion, amino acid supplementation, protein hydrolysates, leucine, arginine, phenylalanine, glutamine, healthy men

INTRODUCTION

The synergistically stimulating effect of the combined intake of carbohydrates and protein on plasma insulin concentrations was described for the first time in the 1960s (1, 2) and was confirmed later by Nuttall et al (3, 4). The insulinotropic effect of intravenous amino acid administration was also studied in the 1960s by Floyd et al (5-10). Infusion of several amino acids led to significant increases in plasma insulin. A mixture of 10 amino acids and equimolar quantities of arginine or leucine only were found to be the most potentiating. Floyd et al also observed a synergistic effect when glucose was administered intravenously with these amino acids. After different combinations of amino acids were investigated, the combined intravenous administration of arginine-leucine and arginine-phenylalanine, together with glucose, resulted in the largest increase in plasma insulin concentrations. Several in vitro studies using incubated β-cells of the pancreas showed strong insulinotropic effects of arginine, leucine, phenylalanine, and leucine in combination with glutamine (11-20).

A strong elevation of plasma insulin concentrations after the ingestion of carbohydrates in combination with a highly insulinotropic amino acid and protein mixture could be of experimental as well as practical importance. For example, in metabolic research such a mixture could be used as a tool to elevate insulin concentrations in vivo without intravenous insulin administration. In patients with type 2 diabetes, the mixture could be used as a means of evaluating the responsiveness of the pancreas. Although more research should be performed, such a mixture could possibly also be of use as a nutritional insulinotropic supplement during the early stages of declining insulin sensitivity in type 2 diabetes, potentially postponing patients' dependency on exogenous insulin administration. In sports nutrition, the addition of an insulinotropic amino acid and protein mixture to carbohydrate drinks could represent a means of increasing postexercise glycogen synthesis rates, as was shown by Zawadzki et al (21), and was investigated by us in another study (22).

Currently there is no literature available that provides clear insight into the type, combination, and quantity of free amino acids or protein sources that should be taken orally in combination

TABLE 1
Composition of the 10 test drinks

Test drink	1	2	3	4	5	6	7	8	9	10
					g/L					
Arginine	—	57.1	19.0	14.3	—	—	—	—	—	9.50
Glutamine	—	—	—	14.3	—	—	—	—	—	—
Leucine	—	—	19.0	14.3	—	—	—	—	14.3	9.50
Phenylalanine	—	—	19.0	14.3	—	—	—	—	14.3	9.50
Whey hydrolysate	—	—	—	—	57.1	—	—	—	—	—
Pea hydrolysate	—	—	—	—	—	57.1	—	—	—	—
Wheat hydrolysate	—	—	—	—	—	—	57.1	—	28.6	28.6
Casein (milk protein)	—	—	—	—	—	—	—	57.1	—	—
Glucose	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1
Maltodextrin	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1	57.1
Sodium saccharinate	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Citric acid	1.80	1.80	1.80	1.80	1.80	1.80	1.801	1.80	1.80	1.80
Cream vanilla flavor	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

with carbohydrates to maximize the in vivo insulin response. The aim of this study was to define an amino acid and protein (hydrolysate) mixture with an optimal insulinotropic effect when added to a carbohydrate drink. A total of 10 drinks were tested in healthy subjects after an overnight fast to investigate the insulinotropic potential of several free amino acids, protein hydrolysates, and an intact protein. Because it was our aim also to investigate the efficiency of such an insulinotropic mixture in increasing postexercise muscle glycogen synthesis (22), similar amounts of carbohydrate and of the amino acid or protein (hydrolysate) mixture were administered, as used previously by Zawadzki et al (21). The choice of the free amino acid compositions tested in this study was based mainly on the outcome of studies by Floyd et al (5–10).

SUBJECTS AND METHODS

Subjects

Eight healthy, nonobese male subjects [$\bar{x} \pm$ SE age: 21 ± 0.4 y; weight: 73.9 ± 2.2 kg; height: 186 ± 2 cm; BMI (in kg/m^2): 21.4 ± 0.7] participated in this study. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. The study was approved by the Ethical Committee of the Academic Hospital Maastricht.

Experimental trials

Each subject participated in 10 trials, separated by ≥ 3 d, in which 10 different beverages were tested. All studies lasted 2 h, during which the subjects were seated and remained inactive. In the initial part of the study, test drinks 1 to 8 (*see* below) were tested. Beverages 9 and 10 were composed and tested within 3 wk after analysis of the acquired data on test drinks 1–8. In both parts of the study, beverages were provided in a random, double-blind fashion. All drinks were vanilla flavored so that the taste would be similar in all trials. The subjects were instructed to refrain from any sort of heavy physical labor and to keep their diets as constant as possible the day before the trials. The subjects had to fast for 12 h before reporting to the laboratory; during that period, the subjects were allowed to drink water or tea (without sugar).

Protocol

The subjects reported to the laboratory at 0830 after an overnight fast. A polytetrafluoroethylene catheter (Quick-Cath; Baxter Healthcare SA, Swinford, Ireland) was inserted into an antecubital vein and a resting blood sample was drawn at time zero. Immediately thereafter, the subjects drank an initial bolus (3.5 mL/kg) of a given test drink. Repeated boluses (3.5 mL/kg) were taken every 30 min for 90 min. Blood samples were drawn at 15-min intervals for measurement of plasma glucose and insulin concentrations. Amino acid concentrations were measured in blood samples taken at 0, 60, and 120 min.

Beverages

At 0, 30, 60, and 90 min, the subjects received a beverage volume of 3.5 mL/kg to ensure a given dose of 0.8 g carbohy-

TABLE 2
Amino acid composition of hydrolysates and intact casein protein

Amino acid	Whey	Pea	Wheat	Casein
		% by wt		
L-Alanine	4.7	3.8	1.8	3.1
L-Cysteine	1.2	0.4	0.9	0.4
L-Aspartate	5.4	4.4	0.2	3.7
L-Glutamate	9.1	7.4	3.2	11.2
L-Phenylalanine	2.4	3.2	4.8	5.4
L-Glycine	1.6	2.8	2.8	1.9
L-Histidine	1.6	1.7	1.6	3.2
L-Isoleucine	5.1	2.4	2.6	5.8
L-Lysine	8.4	5.9	—	8.3
L-Leucine	8.7	5.1	5.6	10.1
L-Methionine	1.3	0.6	1.1	3.0
L-Asparagine	4.4	3.8	1.9	3.7
L-Proline	5.9	2.8	12.3	10.5
L-Glutamine	7.4	6.6	29.0	11.2
L-Arginine	2.0	6.9	2.2	3.8
L-Serine	5.1	4.0	4.4	6.3
L-Threonine	6.6	2.8	2.0	4.6
L-Valine	4.5	2.7	3.0	7.4
L-Tryptophan	1.2	—	—	1.4
L-Tyrosine	2.3	2.6	2.5	5.8

FIGURE 1. Mean (±SE) plasma glucose concentrations after ingestion of the control drink and drinks containing free amino acids (A), the control drink and drinks containing hydrolysates and an intact protein (B), and the control drink and drinks containing mixtures of hydrolysate and free amino acids (C). n = 8. For the exact compositions of the different drinks, see Tables 1 and 2.

drate/kg (50% as glucose and 50% as maltodextrin) and 0.4 g/kg of an amino acid and protein (hydrolysate) mixture every hour. The compositions of all test drinks are listed in **Table 1**. Glucose and maltodextrin were obtained from AVEBE (Veendam, Netherlands), crystalline amino acids were obtained from BUFA (Uitgeest, Netherlands), protein hydrolysates were prepared by Quest (Naarden, Netherlands), and sodium-casein was obtained from DMV (Veghel, Netherlands). Amino acid profiles of the protein hydrolysates and the intact protein tested were provided by the manufacturers and are listed in **Table 2**. All test drinks were uniformly flavored by adding 0.8 g sodium-saccharinate solution (25%, by wt), 3.6 g citric acid solution (50%, by wt), and 5 g of a cream vanilla flavor (Quest) for each 1-L drink.

Analysis

Blood (4 mL) was collected in EDTA-containing tubes and centrifuged at 1000 × g and 4°C for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −40°C. Glucose (Uni Kit III, 07367204; La Roche, Basel, Switzerland) was analyzed with the Cobas Fara semiautomatic analyzer (Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Insulin RIA 100 kit; Pharmacia, Uppsala, Sweden). Plasma (200 μL) for amino acid analysis was deproteinized on ice with 50% (wt:vol) 5-sulfosalicylic acid and mixed, and the clear supernate obtained after centrifugation was stored at −80°C until analyzed. Amino acids were analyzed on an automated dedicated amino acid analyzer (LC 5001; Biotronik, München,

FIGURE 2. Mean (±SE) total 2-h plasma glucose responses (area under the curve; AUC) to each test drink. n = 8. *Significantly different from drink 3. P < 0.05. For the exact compositions of the different drinks, see Tables 1 and 2.

FIGURE 3. Mean (±SE) 2nd-h plasma glucose response (area under the curve; AUC) to each test drink. n = 8. For the exact compositions of the different drinks, see Tables 1 and 2.
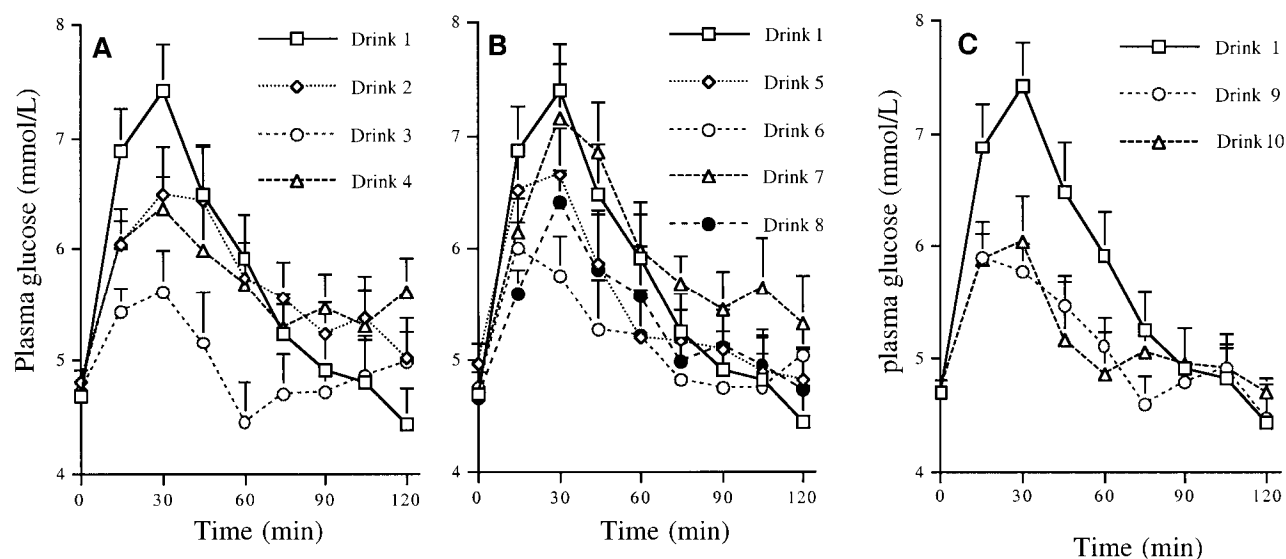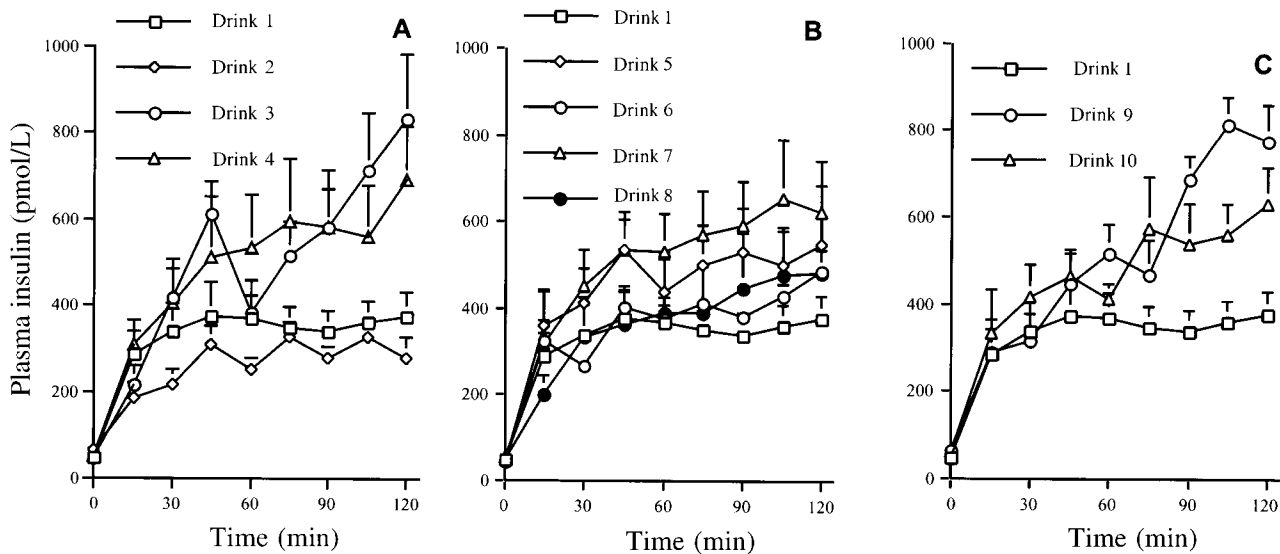
FIGURE 4 Mean (±SE) plasma insulin concentrations after ingestion of the control drink and drinks containing free amino acids (A), the control drink and drinks containing hydrolysates and an intact protein (B), or the control drink and drinks containing mixtures of hydrolysate and free amino acids. $n = 8$. For the exact compositions of the different drinks, *see* Tables 1 and 2. To convert from pmol/L to mU/L, divide by 7.25.

Germany) with use of a cationic exchange resin (type BTC2710, Biotronik), a gradient of lithiumcitrate elution buffers, and post-column derivatization with ninhydrin, all according to working recipes of the suppliers. The same procedures were performed to determine the amino acid composition of the protein (hydrolysates), except that a different amino acid analyzer was used (Pharmacia LKB Biotechnology, Roosendaal, Netherlands). Calibration curves of the amino acids were obtained by using commercial amino acid mixtures. Norvaline was used as internal standard and added to all plasma samples before deproteinization.

Questionnaires

After ingesting the beverage at 60 min and at the completion of each trial, the subjects were asked to fill out a questionnaire that contained questions about gastrointestinal discomfort and other complaints at that time. The presence of nausea, bloated feeling, belching, gastrointestinal cramping, vomiting, diarrhea, urge to defecate, urge to urinate, headache, and dizziness was scored on a 10-point scale (1, absent; 10, strongly present).

Statistics

All data are expressed as means ± SEMs ($n = 8$). The plasma glucose, insulin, and amino acid responses were calculated as area under the curve above baseline value (at 0 min). Statistical analysis of the data was performed by using a 2-factor (treatment and subjects) repeated-measures analysis of variance (ANOVA). Differences between drinks were tested for significance by using Tukey's post hoc test. In addition, simple regression analysis was performed to calculate correlations between the insulin response and the different plasma amino acid responses. Significance was set at $P < 0.05$.

RESULTS

Ingestion of all drinks resulted in an increase in plasma glucose concentrations during the first 30 min, after which concentrations

decreased and stabilized during the second hour (**Figure 1**). After the glucose response was expressed as area under the curve (above baseline) during the entire 2-h trial, significant differences were found between drinks 3 and 7 (**Figure 2**; arginine, leucine, and phenylalanine and wheat protein hydrolysate, respectively). When we focused on the second hour, we found no significant differences in glucose response between the trials (**Figure 3**).

From baseline onward, plasma insulin concentrations strongly increased for the first 30–45 min. After this strong initial increase, insulin concentrations reached a plateau in trials 1 and 2, ie, the control (carbohydrate only) and arginine drinks, respectively (**Figure 4A**). Plasma insulin concentrations after the ingestion of drinks 5, 6, 7, and 8 increased more and leveled off between 45 and 60 min (Figure 4B). Insulin concentrations after ingestion of free amino acid–containing drinks 3, 4, 9, and 10 continued to increase during the last 30 min (Figure 4, A and C). After we expressed the insulin response as the area under the curve (above baseline) during the entire 2-h period, we found significant differences between drink 2 and drinks 3, 4, 7, and 9 (126%, 124%, 122%, and 105% greater than with drink 2, respectively) (**Figure 5**). Compared with the control trial, mean differences as large as 57%, 55%, 54%, and 42%, respectively were found (not significant). Because of the large intersubject variability during the first 45–60 min, caused by differences in gastric emptying and absorption rates, insulin responses were calculated over the second hour. During the second hour plasma insulin responses measured during the administration of drinks 3 and 9 were 2-fold higher than with the carbohydrate-only drink (101% and 103% greater, respectively) (**Figure 6**). Insulin responses after ingestion of drinks 3, 4, 7, and 9 were also significantly higher than after ingestion of the arginine drink (Figure 6). Insulin concentrations at 120 min were substantially higher after ingestion of drinks 3 and 9 than after ingestion of the control drink (122% and 106% greater, respectively).

Mean plasma amino acid concentrations at baseline, 60 min, and 120 min are reported in **Tables 3, 4,** and **5**. Plasma amino
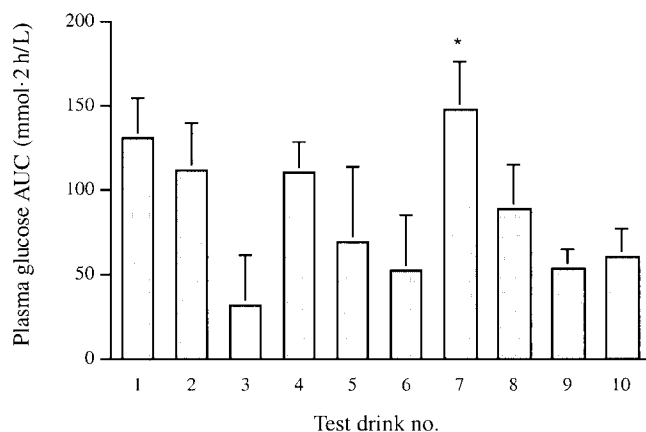
FIGURE 5. Mean (±SE) total 2-h plasma insulin response (area under the curve; AUC) to each test drink. $n = 8$. *Significantly different from drink 2, $P < 0.05$. For the exact compositions of the different drinks, see Tables 1 and 2.
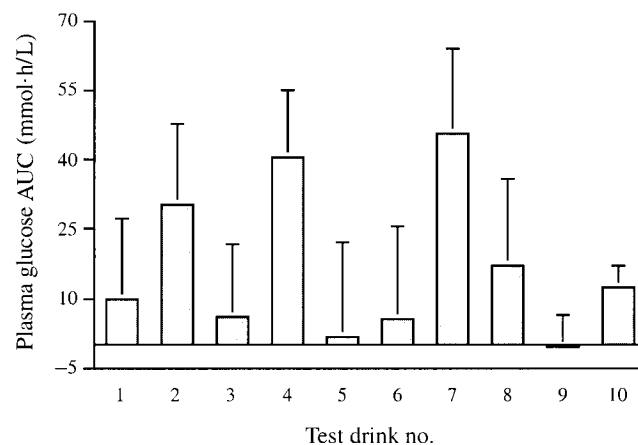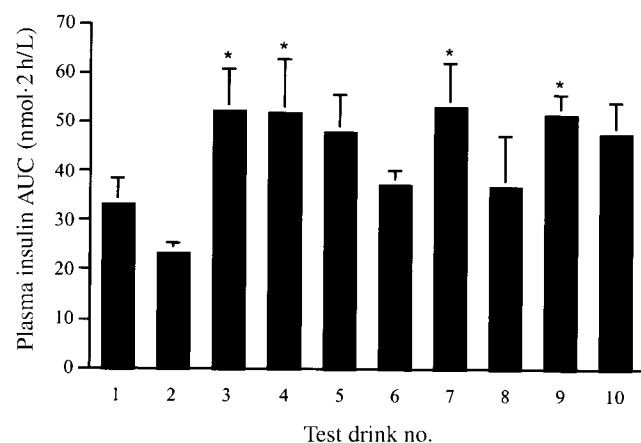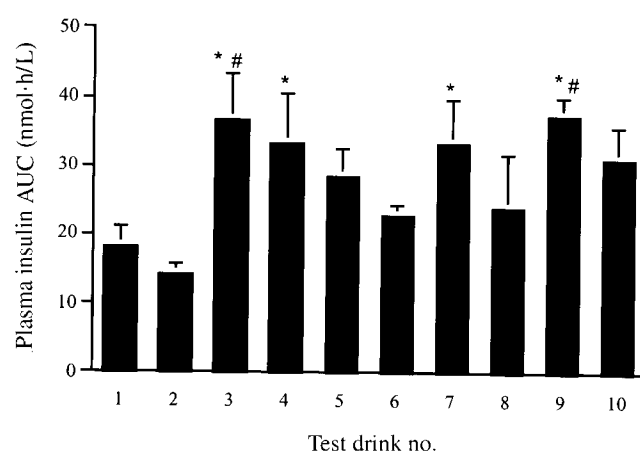
FIGURE 6. Mean (±SE) 2nd-h plasma insulin response (area under the curve; AUC) to each test drink. $n = 8$. #Significantly different from drink 1 (control), $P < 0.05$. *Significantly different from drink 2, $P < 0.05$. For the exact compositions of the different drinks, see Tables 1 and 2.

acid responses were calculated as area under the curve above baseline values. A complete overview of the plasma amino acid responses and the significant differences between the ingestion of the various mixtures is provided in **Table 6.** Only the findings most relevant to the aim of this study are discussed below. Plasma leucine, phenylalanine, and arginine concentrations increased significantly more after ingestion of drink 3 than after ingestion of all other drinks and in parallel showed one of the highest

insulin responses. Ingestion of the other free amino acid–containing drinks (4, 9, and 10) also resulted in significantly higher plasma leucine and phenylalanine responses than did ingestion of the other drinks (1, 2, 5, 6, 7, and 8). Although the amount of free arginine administered in drink 2 was substantially larger than that provided in drinks 3, 4, and 10, the increase in plasma arginine concentrations was significantly lower after ingestion of drink 2 than after ingestion of drinks 3, 4, and 10. Addition of the

TABLE 3
Plasma amino acid concentrations after ingestion of drinks 1–4 containing amino acids and carbohydrate[1]

	Drink 1 (control)			Drink 2[2]			Drink 3[3]			Drink 4[4]		
	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min
						$\mu mol/L$						
Threonine	102± 7	90± 6	82± 6	108± 2	99± 6	87± 3	103± 5	85± 6	64± 4	93± 5	77± 5	61± 4
Serine	91± 5	81± 6	70± 5	98± 6	88± 7	75± 5	90± 8	73± 7	55± 6	87± 7	72± 6	57± 5
Asparagine	52± 4	46± 4	42± 3	49± 2	44± 4	38± 2	50± 3	40± 3	31± 2	48± 3	39± 3	31± 3
Glutamate	78± 10	83± 9	76± 10	124± 11	111± 9	127± 5	110± 16	85± 10	73± 10	115± 9	76± 8	80± 8
Glutamine	667± 32	624± 21	600± 28	631± 29	597± 32	566± 11	578± 25	601± 31	556± 14	559± 28	642± 29	616± 30
Proline	147± 13	134± 14	128± 15	162± 18	164± 25	154± 19	174± 21	144± 18	124± 18	160± 21	128± 20	112± 19
Glycine	217± 16	201± 15	192± 14	228± 11	203± 9	198± 11	221± 12	175± 10	142± 8	218± 15	174± 14	148± 15
Alanine	291± 25	302± 21	306± 18	334± 34	314± 32	350± 20	324± 31	297± 23	271± 17	296± 24	296± 20	284± 18
Citrulline	28± 2	16± 1	12± 1	30± 1	26± 2	20± 2	28± 2	30± 2	29± 2	28± 1	33± 2	40± 3
α-Aminobutyrate	21± 2	20± 2	19± 1	21± 3	20± 3	18± 3	18± 3	17± 3	15± 1	21± 2	19± 2	16± 1
Valine	224± 7	195± 7	169± 5	247± 5	219± 6	185± 2	236± 9	194± 7	118± 5	218± 6	177± 6	111± 5
Methionine	24± 1	20± 1	16± 1	23± 1	20± 1	15± 1	22± 1	19± 1	10± 0	21± 1	16± 1	9± 1
Isoleucine	64± 1	48± 2	33± 2	67± 4	53± 3	30± 2	62± 3	40± 2	11± 1	63± 2	41± 3	11± 1
Leucine	121± 4	90± 6	67± 4	131± 3	108± 6	73± 3	116± 5	568± 48	804± 32	117± 4	502± 37	584± 67
Tyrosine	58± 3	51± 4	44± 3	61± 3	52± 4	42± 3	55± 3	99± 8	131± 12	50± 2	96± 7	167± 44
Phenylalanine	57± 3	47± 4	41± 3	67± 2	57± 3	49± 2	61± 2	407± 32	681± 22	60± 2	328± 25	473± 69
Tryptophan	44± 3	39± 3	33± 3	45± 2	43± 3	37± 2	40± 2	34± 2	23± 2	36± 2	30± 1	37± 17
Ornithine	47± 3	42± 5	36± 3	53± 3	87± 8	163± 15	54± 5	143± 11	260± 24	50± 6	125± 11	197± 21
Lysine	155± 9	143± 10	133± 8	168± 6	164± 12	153± 9	161± 7	181± 11	158± 8	143± 7	149± 8	124± 15
Histidine	73± 2	67± 1	63± 2	79± 3	72± 4	65± 3	72± 2	62± 3	47± 2	70± 2	61± 3	52± 2
Arginine	76± 5	66± 4	58± 4	83± 2	164± 8	286± 19	79± 3	371± 25	538± 29	75± 3	299± 22	410± 24

[1] $\bar{x}$ ± SEM; $n = 8$. For compositions of drinks 1–4, see Tables 1 and 2.
[2] Arginine.
[3] Leucine, phenylalanine, and arginine.
[4] Leucine, phenylalinine, arginine, and glutamine.

TABLE 4
Plasma amino acid concentrations after ingestion of drinks 5–8 containing protein (hydrolysate) and carbohydrate[1]

	5 (whey)			6 (pea)			7 (wheat)			8 (casein)		
	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min	0 min	60 min	120 min
						$\mu mol/L$						
Threonine	118± 7	217± 12	258± 16	113± 6	166± 9	185± 8	109± 7	138± 12	136± 12	113± 6	126± 6	125± 6
Serine	101± 7	150± 11	155± 13	101± 8	141± 9	153± 9	98± 7	134± 8	136± 12	98± 10	109± 11	102± 10
Asparagine	46± 3	81± 6	91± 7	54± 4	98± 8	115± 8	52± 2	62± 4	58± 4	50± 2	57± 3	57± 4
Glutamate	56± 5	62± 5	55± 4	63± 9	60± 9	69± 10	104± 10	86± 8	72± 11	49± 11	42± 6	43± 8
Glutamine	756± 25	815± 29	816± 36	663± 25	725± 30	727± 32	628± 23	754± 21	769± 22	721± 29	722± 23	700± 27
Proline	174± 21	265± 24	306± 25	173± 20	226± 25	252± 22	181± 20	327± 24	393± 31	175± 19	220± 23	245± 20
Glycine	244± 16	239± 14	254± 20	238± 8	285± 12	307± 14	231± 11	260± 14	273± 16	237± 14	229± 14	224± 15
Alanine	305± 25	462± 17	489± 14	326± 30	457± 26	488± 22	317± 19	425± 25	438± 17	353± 30	382± 19	396± 14
Citrulline	34± 2	30± 2	33± 2	32± 2	27± 2	30± 2	32± 2	37± 3	42± 4	31± 2	26± 2	25± 2
α-Aminobutyrate	30± 3	34± 5	36± 4	18± 2	19± 2	18± 2	19± 2	21± 2	19± 2	17± 2	17± 2	16± 2
Valine	254± 10	366± 11	403± 8	241± 3	312± 7	344± 7	243± 6	293± 8	294± 8	248± 8	268± 8	262± 6
Methionine	25± 1	40± 1	47± 2	24± 1	23± 1	18± 1	24± 1	31± 1	30± 1	24± 1	27± 1	27± 1
Isoleucine	69± 4	155± 5	185± 7	63± 2	107± 4	121± 4	70± 2	98± 3	95± 5	72± 3	81± 3	79± 3
Leucine	131± 5	253± 6	294± 8	122± 3	191± 6	213± 7	125± 4	181± 7	188± 9	123± 4	134± 4	127± 4
Tyrosine	59± 4	81± 4	90± 5	57± 2	82± 4	98± 5	58± 4	81± 5	93± 6	55± 2	61± 3	62± 3
Phenylalanine	64± 3	80± 3	81± 2	59± 2	82± 3	93± 2	60± 3	85± 2	97± 3	60± 2	66± 2	66± 2
Tryptophan	50± 3	71± 4	76± 4	52± 2	49± 2	47± 3	46± 3	50± 3	48± 3	48± 2	50± 2	45± 3
Ornithine	55± 5	72± 5	77± 5	56± 5	89± 7	108± 7	54± 4	71± 4	79± 6	59± 8	63± 7	62± 7
Lysine	178± 10	323± 10	374± 10	175± 6	299± 12	348± 12	171± 8	174± 12	159± 10	166± 9	192± 8	200± 8
Histidine	80± 3	94± 4	93± 3	78± 4	93± 5	94± 4	80± 3	96± 3	98± 3	77± 4	81± 5	81± 4
Arginine	83± 5	107± 4	113± 4	82± 4	163± 11	196± 13	82± 4	103± 5	107± 6	83± 3	86± 3	83± 4

[1] $\bar{x}$ ± SEM; n = 8. For composition of drinks 5–8, see Tables 1 and 2.

free amino acids to a wheat protein hydrolysate in trials 9 and 10 clearly resulted in significantly higher plasma leucine and phenylalanine responses than did ingestion of the protein (hydrolysates) in drinks 5, 6, 7, and 8. In general, the ingestion of the intact protein resulted in less marked increases in various plasma amino acid concentrations within the 2-h period than did the ingestion of the protein hydrolysates. More detailed information is provided in Tables 3–6.

TABLE 5
Plasma amino acid concentrations after ingestion of drinks 9 and 10 containing amino acid or protein hydrolysate and carbohydrate[1]

	9 (leu/phe and wheat)			10 (leu/phe/arg and wheat)		
	0 min	60 min	120 min	0 min	60 min	120 min
			$\mu mol/L$			
Threonine	104± 6	102± 6	87± 6	107± 6	111± 8	99± 8
Serine	96± 8	99± 10	84± 9	99± 8	103± 9	89± 9
Asparagine	52± 2	47± 2	39± 3	53± 6	47± 3	39± 3
Glutamate	71± 11	62± 4	68± 7	56± 8	48± 4	42± 5
Glutamine	673± 12	702± 12	686± 29	702± 27	750± 26	740± 18
Proline	174± 22	207± 27	216± 21	193± 32	250± 32	257± 31
Glycine	239± 13	217± 12	197± 13	232± 11	216± 10	203± 13
Alanine	338± 21	346± 24	337± 20	316± 24	362± 18	369± 15
Citrulline	33± 2	37± 3	43± 3	33± 2	41± 3	49± 3
α-Aminobutyrate	15± 1	14± 1	11± 1	18± 2	17± 2	14± 2
Valine	241± 7	234± 5	180± 6	247± 10	245± 8	188± 7
Methionine	22± 1	21± 1	16± 1	23± 1	25± 1	19± 1
Isoleucine	68± 2	65± 2	38± 2	68± 4	70± 4	39± 2
Leucine	122± 4	518± 37	654± 43	122± 4	455± 21	542± 20
Tyrosine	54± 3	112± 9	141± 10	53± 2	114± 8	150± 13
Phenylalanine	61± 3	342± 34	526± 37	60± 1	270± 19	411± 19
Tryptophan	46± 2	42± 2	32± 2	43± 2	43± 2	35± 2
Ornithine	57± 5	65± 6	70± 6	58± 5	123± 8	175± 14
Lysine	169± 11	164± 12	147± 14	160± 7	177± 9	155± 11
Histidine	77± 2	76± 2	69± 2	78± 4	79± 4	70± 3
Arginine	83± 3	94± 4	95± 5	80± 3	289± 15	370± 20

[1] $\bar{x}$ ± SEM; n = 8. Leu, leucine; phe, phenylalanine; arg, arginine. For composition of drinks 9–10, see Tables 1 and 2.

TABLE 6
Plasma amino acid responses after ingestion of drinks containing carbohydrate and amino acid or protein hydrolysate[1]

$mmol \cdot 2h \cdot L^{-1}$

	1 (control)	2 (arg)	3 (leu/phe/arg)	4 (leu/phe/arg/gln)	5 (whey)	6 (pea)	7 (wheat)	8 (casein)	9 (leu/phe and wheat)	10 (leu/phe/arg and wheat)
Threonine	−1.3±0.3[3-5]	−1.0±0.4[2-5]	−2.2±0.2[2-6]	−1.9±0.2[2-6]	10.2±0.7[2-11]	5.4±0.5[2,4-11]	2.5±0.5[3,6-11]	1.2±0.2[3,7-11]	−0.7±0.2[2-5]	0.0±0.2[2-4,9,10]
Serine	−1.2±0.1[2-5]	−1.3±0.3[2-5]	−2.0±0.2[2-6,11]	−1.8±0.2[2-6,11]	4.5±0.4[5-11]	3.9±0.3[5-11]	3.3±0.4[5-11]	0.8±0.2[2-4,7-10]	−0.1±0.2[2-4,9,10]	−0.1±0.1[2-4,9,10]
Asparagine	−0.7±0.1[2-4]	−0.6±0.2[2-3]	−1.2±0.2[2-5]	−1.1±0.1[2-5]	3.5±0.4[6-11]	4.5±0.4[6-11]	0.8±0.2[2,3,6,7,9-11]	0.6±0.1[2-3,9,10]	−0.7±0.1[2-4]	−0.8±0.6[2-4]
Glutamate	0.2±1.1	−0.3±1.0	−2.6±1.1	−3.4±1.1	0.3±0.5	0.0±0.5	−2.1±0.7	−0.6±0.6	−0.6±0.9	−0.9±0.6
Glutamine	−4.6±2.2[2-4,6,10]	−1.6±2.7[3,4,10]	0.7±2.3[4]	6.8±2.2[2,7,8]	5.3±1.4[7]	5.7±2.2[8]	11.8±1.5[2-9,11]	−0.6±1.9[4,10]	2.1±1.3[4]	4.0±1.3[4,7]
Proline	−1.4±0.4[6-11]	0.6±0.9[2-6,9,10]	−3.3±0.7[2-6,8,11]	−3.3±0.4[2-6,8,11]	9.4±0.7[2-11]	5.5±0.6[2,4,7-10]	15.1±1.0[2,3,5-11]	4.0±0.3[2,4,7-10]	3.2±0.5[2,4,7,9,10]	5.3±0.7[2,4,7-10]
Glycine	−1.7±0.5[3,4,9,10]	−1.8±0.6[3,4,9,10]	−5.1±0.6[2-8,11]	−4.7±0.5[2-8,11]	0.0±1.0[2-4,6,9,10]	4.9±0.6[3-5,11]	3.0±0.6[3-5,11]	−0.9±0.3[3,4,9,10]	−2.6±0.4[3,4,9,10]	−1.8±0.5[3,4,9,10]
Alanine	1.1±1.3[4]	1.6±1.9[4]	−3.2±1.4[2-6]	−0.4±1.4[4]	15.0±1.6[5-11]	12.7±1.3[5-11]	10.1±1.4[5-11]	3.0±1.7[2-4,9]	0.4±1.0[2-4]	4.3±1.2[2-4,9]
Citrulline	−1.2±0.1[2-6,8-11]	−0.6±0.1[4,6,7,9-11]	0.1±0.1[4-8,10,11]	0.7±0.1[2,3,5-7,9-11]	−0.3±0.1[4,6,7,10,11]	−0.3±0.1[4,6,7,10,11]	0.6±0.2[2,3,5,7-9]	−0.5±0.1[4,6-11]	0.6±0.2[2,3,5,7-9]	1.0±0.2[2,3,5,7-9]
α-Aminobutyrate	−0.1±0.1[2]	−0.2±0.1[2]	−0.2±0.2[2]	−0.3±0.1[2]	0.4±0.2[6-11]	0.1±0.1	0.1±0.0	0.0±0.0	−0.2±0.0[2]	−0.2±0.0[2]
Valine	−3.4±0.4[2-5,9,10]	−3.4±0.4[2-5,9,10]	−6.1±0.5[2-8,11]	−5.7±0.4[2-8,11]	11.2±0.7[2-11]	7.3±0.6[2-4,11]	4.5±0.4[2-3,5-11]	1.7±0.3[2-4,6-11]	−2.3±0.4[2-5,9,10]	−1.9±0.5[2-5,9,10]
Methionine	−0.5±0.1[2-4,6]	−0.4±0.1[2,4,5]	−0.5±0.1[2-4,6]	−0.6±0.1[2-4,6,11]	1.6±0.1[3-11]	−0.3±0.1[2,4,5]	0.6±0.1[2,3,6-11]	0.3±0.1[2,3,7-11]	−0.2±0.1[2-4,5,10]	0.0±0.1[2-4,7,9,10]
Isoleucine	−1.9±0.2[2-6]	−2.2±0.2[2-6]	−2.8±0.2[2-6,11]	−2.9±0.1[2-6,11]	8.7±0.4[3-11]	4.4±0.4[2-4,11]	2.5±0.3[2-3,5-11]	0.7±0.1[2-4,6-11]	−1.1±0.1[2-5,9,10]	−0.8±0.2[2-5,7-10]
Leucine	−3.4±0.3[4-6,9-11]	−3.5±0.4[4-6,9-11]	47.7±3.7[2-8,10,11]	37.1±3.1[2-5,7-9]	12.2±0.6[5-11]	6.9±0.7[6-11]	5.3±0.5[9-11]	0.8±0.2[2-6,9-11]	39.7±3.4[2-5,7-9]	32.6±1.7[2-5,7-9]
Tyrosine	−0.8±0.2[2-4,6,9-11]	−1.1±0.2[2-4,6,9-11]	4.9±0.7[2-5,7,8]	6.3±1.5[2-5,7,8]	2.3±0.2[6-8,10,11]	2.8±0.3[6-8,10,11]	2.4±0.5[6-8,10,11]	0.6±0.2[6,9-11]	6.1±0.8[2-5,7,8]	6.6±0.8[2-5,7,8]
Phenylalanine	−1.1±0.1[6,9-11]	−1.3±0.3[6,9-11]	39.4±2.3[2-8,10,11]	28.5±2.7[2-5,7-9]	1.5±0.1[6,9-11]	2.4±0.2[6,9-11]	2.6±0.1[6,9-11]	0.5±0.2[6,9-11]	30.8±3.2[2-9]	23.1±1.6[2-5,7-9,11]
Tryptophan	−0.6±0.1[2]	−0.1±0.1	−0.9±0.1[2,4]	−0.3±0.6[2]	2.0±0.2[2-7,9-11]	−0.3±0.2[2]	0.2±0.1[2,9]	0.1±0.1[2]	−0.6±0.1[2]	−0.2±0.2[2]
Ornithine	−0.6±0.1[2-4,6,8-10]	7.7±0.6[2-5,7,9,11]	11.6±0.9[2-8,10,11]	8.9±0.8[2-5,7,9,11]	1.7±0.1[3,6-10]	3.5±0.3[2-5,11]	1.8±0.2[6-10]	0.4±0.2[3,6,8-10]	0.9±0.2[3,6,8-10]	7.4±0.5[2-5,7-9,11]
Lysine	−1.4±0.4[2,3,5,9]	0.0±0.6[2,3,5,9]	1.1±0.6[2,3,7,8]	−0.2±0.6[3,5]	14.5±0.5[4-11]	12.6±0.9[4-11]	−0.2±0.5[3,3,5]	2.6±0.2[2-4,7,8,10,11]	−1.0±0.2[2,3,5]	0.9±0.3[2,3]
Histidine	−0.7±0.0[2-5,9]	−0.7±0.2[2-5]	−1.4±0.2[2-7,11]	−1.1±0.1[2-6,11]	1.2±0.2[5-11]	1.4±0.2[5-11]	1.6±0.2[5-11]	0.4±0.1[2-4,7-11]	−0.3±0.1[2-5,9,10]	−0.2±0.1[2-4,9,10]
Arginine	−1.2±0.2[3,6,8-10]	16.5±0.8[2-7,9-11]	31.3±1.9[2-8,10,11]	23.5±1.5[2-5,7-9,11]	2.3±0.3[3,6,8-10]	8.2±0.8[2-4,11]	2.0±0.4[3,6,8-10]	0.2±0.1[3,6,8-10]	1.0±0.3[3,6,8-10]	21.2±1.4[2-5,7-9,11]

[1] Plasma amino acid response expressed as area under the curve minus baseline values. $\bar{x} \pm$ SEM; $n = 8$. For composition of drinks 1–10, see Tables 1 and 2 Arg, arginine; leu, leucine; phe, phenalalanine; gln, glutamine.
[2] Significantly different from drink 5, $P < 0.05$.
[3] Significantly different from drink 6, $P < 0.05$.
[4] Significantly different from drink 7, $P < 0.05$.
[5] Significantly different from drink 8, $P < 0.05$.
[6] Significantly different from drink 10, $P < 0.05$.
[7] Significantly different from drink 1, $P < 0.05$.
[8] Significantly different from drink 2, $P < 0.05$.
[9] Significantly different from drink 3, $P < 0.05$.
[10] Significantly different from drink 4 $P < 0.05$.
[11] Significantly different from drink 9, $P < 0.05$.

TABLE 7
Statistical analysis of questionnaire results[1]

Question	Test drink no.									
	1	2	3	4	5	6	7	8	9	10
1 (nausea)	—	—	—	—	—	—	—	—	—	—
2 (bloated feeling)	4	—	—	—	4	—	4	6	—	4
3 (belching)	3	3	—	—	3	—	3	3,4	—	3
4 (gastrointestinal cramps)	—	—	—	—	—	—	—	—	—	—
5 (vomiting)	—	—	—	—	—	—	—	—	—	—
6 (diarrhea)	2	—	2	2	2	2	2	2	2	2
7 (urge to defecate)	2	—	2	2	2	2	2	2	2	2
8 (urge to urinate)	—	—	—	—	—	—	—	—	—	—
9 (headache)	4	—	—	—	—	4	4	4	4	4
10 (dizziness)	—	—	—	—	—	—	—	—	—	—

[1] The numbers within the table represent the drinks with a significantly ($P < 0.05$) higher score for the indicated symptom compared with the drink indicated in the column head.

Strong positive correlations were found between insulin responses and increases in plasma leucine ($P < 0.003$), phenylalanine ($P < 0.02$), tyrosine ($P < 0.0001$), and citrulline ($P < 0.0023$). A significant negative correlation was observed between insulin responses and plasma glutamate ($P < 0.02$) concentrations.

The data derived from the questionnaires were analyzed, and significant differences between drinks are indicated in **Table 7**. Gastrointestinal problems were found after administration of the arginine drink (2). Subjects scored higher for presence of urge to defecate and diarrhea (3.1 and 3.4, respectively) after ingestion of drink 2 than after ingestion of all other drinks (1.2 and 1.0, respectively). Eventually, all subjects experienced severe diarrhea for several hours during and after ingestion of test drink 2 (0.4 g arginine kg body wt$^{-1}\cdot$h^{-1}). This was not observed in trials 3 and 4, in which dosages of 0.13 and 0.10 g arginine·kg body wt$^{-1}\cdot$h^{-1}, respectively, were ingested. Furthermore, significantly higher scores for the presence of headache and bloated feeling were reported after ingestion of the free amino acids in drink 4 (1.6 and 3.3, respectively) than after ingestion of several other test drinks (mean overall score: 1.1 and 1.7, respectively). In addition, a significantly higher score for belching was found after ingestion of the free amino acids in drink 3 (score: 3.4) than after ingestion of several other test drinks (mean overall score: 1.8). These symptoms were absent in the trials that combined those free amino acids with a wheat protein hydrolysate (Table 7).

DISCUSSION

The results of this study indicate that oral ingestion of some amino acid mixtures in combination with carbohydrates can produce strong insulinotropic effects. Four free amino acids (leucine, phenylalanine, arginine, and glutamine) were tested. Several in vitro studies showed that these amino acids have a strong stimulating effect on insulin release by pancreatic β-cells (12–14, 19). Floyd et al (5, 7) observed that 30 g arginine injected intravenously in vivo in human subjects led to an equal insulin response as occurred with the mixture of 10 amino acids (30 g in total; arginine, lysine, phenylalanine, leucine, valine, methionine, histidine, isoleucine, threonine, and tryptophan).

The data in this study show clearly that oral ingestion of large amounts of free arginine (0.4 g arginine·kg body wt$^{-1}\cdot$h^{-1}, as ingested in trial 2) is not an effective means of increasing plasma insulin concentrations (Figure 4A) and plasma arginine concen-

trations (Table 3). Ingestion of drink 2 caused severe diarrhea and the urge to defecate in all subjects for several hours during and after the trial. These gastrointestinal problems appeared to prevent intestinal absorption of the arginine because lower concentrations of arginine were seen in plasma after ingestion of drink 2 than after ingestion of drinks 3, 4, and 10 (ingestion rates of 0.13, 0.10, and 0.07 g arginine·kg body wt$^{-1}\cdot$h^{-1}, respectively). These problems also indicate that in sports practice, ingestion of large amounts of arginine to stimulate growth hormone release and muscle anabolism is not recommended. On the other hand, low doses of arginine (< 2 g), as present in commercial sports supplements, do not increase plasma insulin and growth hormone concentrations (23–25).

In later studies, Floyd et al (8, 9) investigated the combined effect of intravenous administration of glucose with combinations of amino acids and found that arginine-leucine and arginine-phenylalanine resulted in the strongest increase in plasma insulin concentrations. We investigated the insulinotropic effect of oral administration of a mixture of arginine, leucine, and phenylalanine (drink 3). A significantly greater insulin response was seen compared with the control trial (101% greater: $P < 0.05$). The increased plasma insulin concentration was attended by a strong significant increase in plasma arginine, leucine, and phenylalanine concentrations (Tables 3 and 6).

Sener and Malaisse (16) observed that the addition of leucine to the incubation medium stimulates insulin release by pancreatic β-cells in vitro. The mechanism behind this effect was investigated, and it was found that leucine activates glutamate dehydrogenase activity in pancreatic β-cells. This subsequently leads to an increase in tricarboxylic acid cycle activity and oxygen consumption of the pancreatic β-cells and is attended by increased insulin production. The addition of glutamine to the incubation medium potentiates the leucine-induced increase in insulin release by providing substrate for glutamate dehydrogenase, whereas glutamine per se has no effect (16). Consequently, we studied the effect of the addition of glutamine to drink 4. However, we observed no differences in insulin response between trials 3 and 4, suggesting that in humans in vivo, enough glutamine is present (600–800 μmol·L^{-1} in plasma; Table 3) to serve as fuel for the pancreas. Also note that the addition of free glutamine hardly influenced plasma glutamine concentrations. Plasma glutamine responses after the ingestion of drink 4 were in fact not significantly different than after ingestion of most other drinks (drinks 3, 5–7, 9, and 10; Tables 4 and 5). No significant differences were found between

the insulin responses in test trials 5, 6, and 7 and the carbohydrate-only trial. Nonetheless, mean insulin responses were 55%, 25%, and 81% greater, respectively, than those observed in the control trial. There were no differences in plasma leucine and phenylalanine responses between the different protein hydrolysates tested (Tables 4 and 6). None of the hydrolysates was associated with gastrointestinal or other complaints.

To compare the insulinotropic effect of the ingestion of the protein hydrolysates with that of an intact protein, sodium-casein was provided in drink 8. This resulted in an insulin response that was not significantly different from that found with the control trial (30% greater) and tended to be less than the responses observed after ingestion of the protein hydrolysates (drink 5 and 7). After ingestion of the intact protein, plasma amino acid responses over this 2-h period were in general lower than the responses observed after ingestion of the protein hydrolysates (Table 6). We conclude that the use of protein hydrolysates is preferred to stimulate insulin secretion because this results in a faster increase in plasma amino acid concentrations during a 2-h period than does intact protein. Another practical disadvantage of the use of an intact protein when ingested as a drink is that most intact proteins have poor solubility in water.

On the basis of the results obtained after trials 1–8, it was concluded that ingestion of free glutamine is not required to obtain an optimal insulin response, whereas the use of free arginine should be restricted to low doses. It was further concluded that ingestion of relative large quantities of amino acids (arginine, leucine, phenylalanine, and glutamine) can cause gastrointestinal and other complaints (drinks 2, 3, and 4; Table 7). In an attempt to combine gastrointestinal tolerance and palatability with a maximal insulin response, drinks 9 and 10 were prepared. Ingestion of both drinks resulted in large insulin responses. In trial 9, leucine and phenylalanine were ingested in combination with the wheat protein hydrolysate and we observed a larger insulin response (103% greater, $P < 0.05$) than with the carbohydrate-only trial that was similar to the response found in trial 3 but without the occurrence of any gastrointestinal and other complaints. Plasma leucine and phenylalanine responses were higher than with the control, arginine, and protein (hydrolysate) drinks but were lower than the response after the ingestion of drink 3. In trial 10, free arginine was added to this mixture, but this showed no further increase in insulin response (69% greater than with the control trial).

Regression analysis of the insulin responses and the changes in plasma amino acid concentrations over the 2-h period showed a strong positive correlation between the observed insulin response and changes in plasma leucine ($P < 0.003$), phenylalanine ($P < 0.02$), and tyrosine ($P < 0.0001$) concentrations. This agrees with several in vitro studies in which β-cells of the pancreas were incubated with leucine and phenylalanine (11–20) and with the in vivo studies by Floyd et al (5–10) in which amino acids were infused. The correlation observed with tyrosine concentrations may be explained by the fact that tyrosine is formed by the hydroxylation of phenylalanine when large amounts of phenylalanine are ingested (26). As such, tyrosine concentrations were higher in drinks containing large amounts of phenylalanine (Tables 3–5). In addition, we observed an unexplained positive correlation with citrulline ($P < 0.002$) and a negative correlation with glutamate ($P < 0.019$).

The main conclusion is that oral intake of amino acids in combination with carbohydrates can result in an insulinotropic effect as much as 100% greater than with the intake of carbohydrates

only. It was shown that a mixture of free leucine, phenylalanine, and arginine can produce a large insulinotropic effect when ingested in combination with carbohydrates. It was also shown that the addition of leucine and phenylalanine to a (wheat) protein hydrolysate can create a similar insulinotropic effect without any gastrointestinal discomfort. These mixtures should provide a useful tool to strongly elevate plasma insulin concentrations in future metabolic studies in healthy subjects and in patients.

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Human Nutrition and Metabolism

Ingestion of Protein Hydrolysate and Amino Acid–Carbohydrate Mixtures Increases Postexercise Plasma Insulin Responses in Men[1]

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ABSTRACT   To optimize the postexercise insulin response and to increase plasma amino acid availability, we studied postexercise insulin levels after the ingestion of carbohydrate and wheat protein hydrolysate with and without free leucine and phenylalanine. After an overnight fast, eight male cyclists visited our laboratory on five occasions, during which a control drink and two different beverage compositions in two different doses were tested. After they performed a glycogen-depletion protocol, subjects received a beverage (3.5 mL · kg^{-1}) every 30 min to ensure an intake of 1.2 g · kg^{-1} · h^{-1} carbohydrate and 0, 0.2 or 0.4 g · kg^{-1} · h^{-1} protein hydrolysate (and amino acid) mixture. After the insulin response was expressed as the area under the curve, only the ingestion of the beverages containing wheat protein hydrolysate, leucine and phenylalanine resulted in a marked increase in insulin response (+52 and + 107% for the 0.2 and 0.4 g · kg^{-1} · h^{-1} mixtures, respectively; $P < 0.05$) compared with the carbohydrate-only trial). A dose-related effect existed because doubling the dose (0.2–0.4 g · kg^{-1} · h^{-1}) led to an additional rise in insulin response ($P < 0.05$). Plasma leucine, phenylalanine and tyrosine concentrations showed strong correlations with the insulin response ($P < 0.0001$). This study provides a practical tool to markedly elevate insulin levels and plasma amino acid availability through dietary manipulation, which may be of great value in clinical nutrition, (recovery) sports drinks and metabolic research.   J. Nutr. 130: 2508–2513, 2000.

KEY WORDS: • *insulin secretion* • *amino acids* • *postexercise recovery* • *leucine* • *phenylalanine* • *humans*

Intensive exercise leads to an increase in muscle protein synthesis (Biolo et al. 1995b, Yarasheski et al. 1999) and muscle protein degradation (Biolo et al. 1995b) in the postexercise phase in humans. Biolo et al. (1997) demonstrated that hyperaminoacidemia, resulting from the intravenous infusion of amino acids, increases postexercise muscle protein synthesis rates and prevents the exercise-induced increase in protein degradation. Recent studies have demonstrated that amino acid ingestion, with (Rasmussen et al. 2000) and without (Tipton et al. 1999) carbohydrates, can also increase postexercise muscle protein synthesis and net protein balance (protein synthesis minus protein degradation). As such, postexercise amino acid ingestion may be an effective method to maximize the anabolic effect of exercise. The mechanisms responsible for this amino acid–induced, anabolic response have not yet been established. Potential regulating factors include changes in levels of various hormones, paracrine substances and vasodilators. Insulin has been proposed as an important factor in protein metabolism, because acute physiologic elevations of plasma insulin levels, especially during conditions of hyperaminoacidemia, result in an additional increase in net muscle protein anabolism in vivo in humans

(Fryburg et al. 1995, Gelfand and Barrett, 1987, Hillier et al. 1998). However, insulin should not be regarded as a primary regulator because in the absence of elevated amino acid concentrations, insulin levels exert only a modest effect on muscle protein synthesis (Biolo et al. 1995a). In accordance, Anthony et al. (1999 and 2000) reported a stimulating effect of leucine ingestion on postexercise muscle protein synthesis in rats independent of an increase in plasma insulin levels.

Insulin also stimulates muscle glucose utilization through the activation of glucose transport (Ivy 1997 and 1998, Ivy and Kuo 1998) and glycogen synthase (Bak et al. 1991, Kruszynska et al. 1986), which is generally considered to be the major factor to determine the rate of glycogen synthesis when substrate supply is adequate (Conlee et al. 1978). Therefore, an increase in postexercise insulin response, after the ingestion of protein (and amino acids) in combination with carbohydrates, has been suggested to accelerate muscle glycogen synthesis (van Hall et al. 2000, van Loon et al. 2000b, Zawadzki et al. 1992). Because of the proposed role of insulin and amino acids in promoting postexercise muscle protein anabolism and/or muscle glycogen synthesis, there is increasing interest in nutritional strategies to maximize postexercise insulin levels and to increase plasma amino acid availability.

In the 1960s, the synergistically stimulating effect of the combined intake of carbohydrate and protein on plasma insu-

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lin levels was first reported (Pallotta and Kennedy 1968, Rabinowitz et al. 1966) and was later confirmed by Nuttall et al. (1984 and 1985). In addition, the infusion of free amino acids increases plasma insulin levels in humans (Fajans et al. 1962, Floyd et al. 1963, 1966, 1968, 1970a, 1970b). We recently studied the effects of the combined oral intake of carbohydrate $(0.8 \ g \cdot kg^{-1} \cdot h^{-1})$ with different amino acids and/or protein (hydrolysates) $(0.4 \ g \cdot kg^{-1} \cdot h^{-1})$ on plasma insulin levels in the postabsorptive resting state and observed a twofold increase in insulin response after the ingestion of carbohydrate with a mixture of wheat protein hydrolysate, free leucine and phenylalanine compared with the intake of only carbohydrate (van Loon et al. 2000a). A synergistic increase in insulin response was also observed after the ingestion of this mixture $(0.4 \cdot kg^{-1} \cdot h^{-1})$ with carbohydrate $(0.8 \ g \cdot kg^{-1} \cdot h^{-1})$ after exercise in trained athletes (van Loon et al. 2000b). In the same study, we observed that the ingestion of this mixture accelerated postexercise muscle glycogen synthesis compared with the ingestion of only carbohydrate $(0.8 \ g \cdot kg^{-1} \cdot h^{-1})$ but not compared with the ingestion of a higher amount of carbohydrate $(1.2 \ g \cdot kg^{-1} \cdot h^{-1})$.

The present study was designed to investigate the effects of the ingestion of different amounts of protein hydrolysate, with and without the addition of free leucine and phenylalanine, in combination with a large amount $(1.2 \ g \cdot kg^{-1} \cdot h^{-1})$ of carbohydrate, on the postexercise insulin response and plasma amino acid concentration in trained athletes. Our aim was to define the mixture with the strongest insulinotropic properties in combination with increased amino acid availability when ingested in the postexercise phase. Theoretically, this mixture would be the best candidate to explore whether such an insulinotropic mixture can maximize postexercise net muscle protein anabolism and glycogen synthesis rates.

SUBJECTS AND METHODS

Subjects. Eight highly trained male cyclists [age 24.0 ± 0.6 y, body mass 70.0 ± 1.0 kg, body mass index 21.4 ± 0.6 kg/m², maximum workload $(W_{max})^3$ 390 ± 8 W, maximum heart rate 191 ± 3 bpm) participated in this study. Subjects trained at least three times a week for 2 h and had a training history of >5 y. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. This study was approved by the local ethics committee.

Pretesting. Maximum oxygen uptake capacity $(\dot{V}O_{2 \ max})$ and W_{max} were measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen, the Netherlands) during an incremental exhaustive exercise test 1 wk before the first experimental trial (Kuipers et al. 1985). These findings were used to determine the power output settings in the glycogen-depletion protocol.

Experimental trials. Each subject participated in five trials, separated by ≥3 d, in which five different beverages were tested. During those trials, subjects first performed a glycogen-depletion protocol (Kuipers et al. 1987). Thereafter, subjects were studied for 3 h while ingesting 1.2 g of carbohydrate $\cdot kg^{-1} \cdot h^{-1}$ (60:40% maltodextrin/glucose). Blood samples were taken at 30-min intervals. During those 3 h, subjects remained physically inactive. Beverages were provided in a random order. Both subjects and researchers who were present were unaware of the specific drink being tested. All drinks were vanilla flavored to make the taste similar among the trials. Subjects were instructed to refrain from heavy physical labor and to keep their diet constant the day before the trials. Subjects fasted for 12 h before reporting to the laboratory but were allowed to drink water ad libitum.

Protocol. Subjects reported to the laboratory at 0830 h. Muscle glycogen depletion was established by performing an intense cycle

ergometer test (Kuipers et al. 1987). This muscle glycogen-depletion protocol started off with a 10-min warm-up period at a workload of 50% W_{max}. Thereafter subjects were instructed to cycle for 2-min block periods at alternating workloads of 90 and 50% W_{max}. This was continued until subjects were no longer able to complete the 2 min at 90% W_{max}. That moment was defined as the inability to maintain cycling speed at 60 rpm. At that moment, the high intensity blocks were reduced to an intensity equal to 80% W_{max}. Again, subjects had to cycle until they were unable to complete a 2-min block at 80% W_{max}, after which the high intensity block was reduced to 70% W_{max}. Subjects were allowed to stop exercising when they were not able to maintain pedaling speed at >60 rpm at this 70% W_{max}. Subjects were allowed to drink up to 1.0 L of water during the depletion test. After cessation of the exercise, subjects were allowed to take a 5-min shower, after which a Teflon catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn (t = 0 min). Immediately thereafter, subjects drank an initial bolus $(3.5 \ mL \cdot kg^{-1})$ of a given test drink. Subjects were seated for the next 3 h, during which they received a beverage volume of $3.5 \ mL \cdot kg^{-1}$ every 30 min until t = 150. Blood samples (4 mL) were taken every 30 min for the measurement of plasma glucose, insulin and amino acids until t = 180.

Beverages. All beverages contained $68.5 \ g \cdot L^{-1}$ glucose, $102.8 \ g \cdot L^{-1}$ maltodextrin, $0.20 \ g \cdot L^{-1}$ sodium saccharinate, $1.80 \ g \cdot L^{-1}$ citric acid and $5.00 \ g \cdot L^{-1}$ vanilla cream flavor. In addition, beverages 2 and 3 contained 28.6 and $57.1 \ g \cdot L^{-1}$ wheat protein hydrolysate, respectively, whereas beverage 4 contained $14.3 \ g \cdot L^{-1}$ wheat protein hydrolysate and $7.1 \ g \cdot L^{-1}$ of both free leucine and phenylalanine. Beverage 5 contained twice the level of wheat protein hydrolysate and free leucine and phenylalanine compared with beverage 4. At t = 0, 30, 60 90, 120 and 150 min, subjects received a beverage volume of $3.5 \ mL \cdot kg^{-1}$ to ensure a given dosage of $1.2 \ g \cdot kg^{-1} \cdot h^{-1}$ carbohydrate (40:60% maltodextrin/glucose) and 0, 0.2 or $0.4 \ g \cdot kg^{-1} \cdot h^{-1}$ of a mixture containing wheat hydrolysate or wheat hydrolysate with the addition of free leucine and phenylalanine. Glucose and maltodextrin were obtained from AVEBE (Veendam, the Netherlands), crystalline amino acids were obtained from BUFA (Uitgeest, the Netherlands) and the protein hydrolysate (Hyprol) was prepared by Quest International (Naarden, the Netherlands). The protein hydrolysate is prepared from wheat protein via enzymatic digestion and has a medium chain length of 11 amino acids. The amino acid profile of the wheat hydrolysate is listed in **Table 1**. The maltodextrin used had a medium chain length of 14–16 glycosyl

TABLE 1

Amino acid profile of the wheat protein hydrolysate

Amino acid	g · 100 g hydrolysate^{-1}
L-Alanine (Ala)	1.8
L-Cysteine (Cys)	0.9
L-Aspartate (Asp)	0.2
L-Glutamate (Glu)	3.2
L-Phenylalanine (Phe)	4.8
L-Glycine (Gly)	2.8
L-Histidine (His)	1.6
L-Isoleucine (Ile)	2.6
L-Lysine (Lys)	...
L-Leucine (Leu)	5.6
L-Methionine (Met)	1.1
L-Asparagine (Asn)	1.9
L-Proline (Pro)	12.3
L-Glutamine (Gln)	29.0
L-Arginine (Arg)	2.2
L-Serine (Ser)	4.4
L-Threonine (Thr)	2.0
L-Valine (Val)	3.0
L-Tryptophan (Trp)	...
L-Tyrosine (Tyr)	2.5

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[3] Abbreviation used: W_{max}, maximal workload.

units. To make the taste comparable in all trials, sodium saccharinate, citric acid and vanilla cream flavor (Quest International) were added.

Analysis. Blood was collected in EDTA-containing tubes and was centrifuged at 1000 × g and 4°C for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −40°C. Glucose (Uni Kit III 07367204; Hoffman-La Roche, Basel, Switzerland) was analyzed with the COBAS FARA semiautomatic analyzer (Hoffman-La Roche). Insulin was analyzed by radioimmunoassay (Insulin RIA 100 kit; Pharmacia, Uppsala, Sweden). Plasma (200 µL) for amino acid analysis was deproteinized on ice with 500 g · L^{-1} 5-sulfosalicylic acid and vortex mixed. The clear supernatant obtained after centrifugation was stored at −80°C until analysis for amino acids. Amino acids were analyzed on an automated dedicated amino acid analyzer (LC5001; Biotronik, München, Germany) using a cationic exchange resin (type BTC2710; Biotronik), a gradient of lithium citrate elution buffers and postcolumn derivatization with ninhydrin, all according to working recipes of the supplier. Same procedures were performed to determine the amino acid composition of the wheat protein hydrolysate except for the use of a different amino acid analyzer (Pharmacia LKB Biotechnology, Roosendaal, the Netherlands). Calibration curves of the amino acids were obtained using commercial amino acid mixtures. Norvaline was used as internal standard and added to all plasma samples before deproteinization.

Statistics. All data are expressed as means ± SEM (n = 8). Analysis of variance for repeated measures was performed to study differences in plasma glucose and insulin concentrations over time between groups. A Scheffé post hoc test was applied in case of a significant F-ratio to locate the differences. The plasma glucose, insulin and amino acid responses were calculated as the area under the curve above baseline value (t = 0 min). Statistical analyses of these data were conducted with a two-factor analysis of variance with treatment and subject as the two factors. Differences between drinks were checked for statistical significance using the Tukey post hoc test. Simple regression analysis was performed to calculate correlations between the insulin response and the different plasma amino acid responses. Statistical significance was set at P < 0.05.

RESULTS

In all trials, plasma glucose concentrations increased during the first 30 min after beverage ingestion, after which they decreased during the remaining 150 min (**Fig. 1**). After expression of the glucose response as the area under the curve (above baseline) during the entire 3-h period, no significant differences were observed between the different test drink trials.

Plasma insulin concentrations increased in all trials during the first 150 min. In the final 30 min, a plateau developed (**Fig. 2**). The ingestion of drink 5 resulted in significantly higher insulin levels at t = 60, 90, 120 and 150 min compared with drinks 1, 2 and 3. After expression of the insulin response as the area under the curve during the entire 3-h period (**Fig. 3**), insulin responses after the ingestion of drinks 4 and 5 were significantly higher than control (+52 ± 10 and +107 ± 17%, respectively; P < 0.05). The ingestion of drinks 2 and 3 did not result in significantly higher postexercise insulin responses compared with the control drink, and responses were significantly lower compared with the insulin responses reported after the ingestion of the free amino acid–containing drinks (drinks 4 and 5). In addition, the ingestion of drink 5 resulted in a significantly higher insulin response (+36 ± 6%; P < 0.05) compared with drink 4, in which a lower dose of the same mixture was ingested (0.4 versus 0.2 g · kg^{-1} · h^{-1}, respectively).

Plasma amino acid responses were calculated as the areas under the curve above baseline values (**Table 2**). Only the findings most relevant for the aim of this study are reported here. After the postexercise ingestion of the control drink, a decrease was seen in the concentration of all amino acids. The
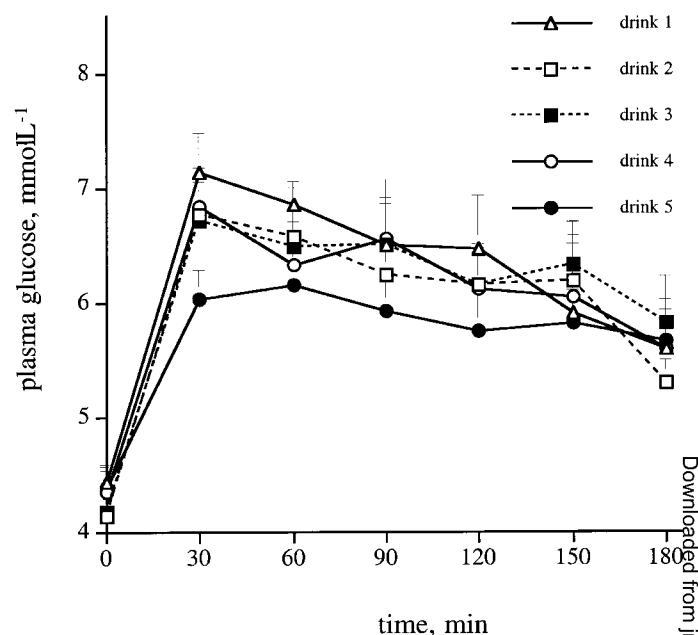

FIGURE 1 Postexercise plasma glucose concentrations after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM (n = 8). No significant differences between trials, P < 0.05.

ingestion of drinks 2 and 3 (wheat) resulted in a significantly higher plasma concentration for most amino acids measured compared with the control. The ingestion of drinks 4 and 5 resulted in a substantial increase in plasma leucine, phenylalanine and tyrosine responses compared with the other drinks (Table 2). Regression analysis revealed a strong positive correlation between the size of the insulin response and the change in plasma leucine (P < 0.0001, r = 0.66), phenylalanine (P < 0.0001, r = 0.62) and tyrosine (P < 0.0001, r = 0.57) concentrations. Plasma threonine, asparagine, glycine, alanine, valine, methionine, isoleucine and histidine responses showed a negative correlation with the insulin response (P < 0.05, r = −0.33 to −0.48) within this postexercise setting.

DISCUSSION

It was recently concluded that the ingestion of beverages containing protein hydrolysate plus carbohydrate is preferred over the ingestion of those containing intact protein plus carbohydrate to stimulate insulin secretion and plasma amino acid availability, because ingestion results in a stronger increase in plasma amino acid levels in the postabsorptive resting state (van Loon et al. 2000a). In addition, the use of an intact protein when ingested as a beverage has another practical disadvantage because most intact proteins are poorly soluble in water. In an attempt to combine gastrointestinal tolerance and palatability with a maximal insulin response, a mixture of wheat hydrolysate with free leucine and phenylalanine was defined (van Loon et al. 2000a). The insulinotropic properties after the ingestion of this mixture in the postabsorptive resting state exceeded those of most other combina-
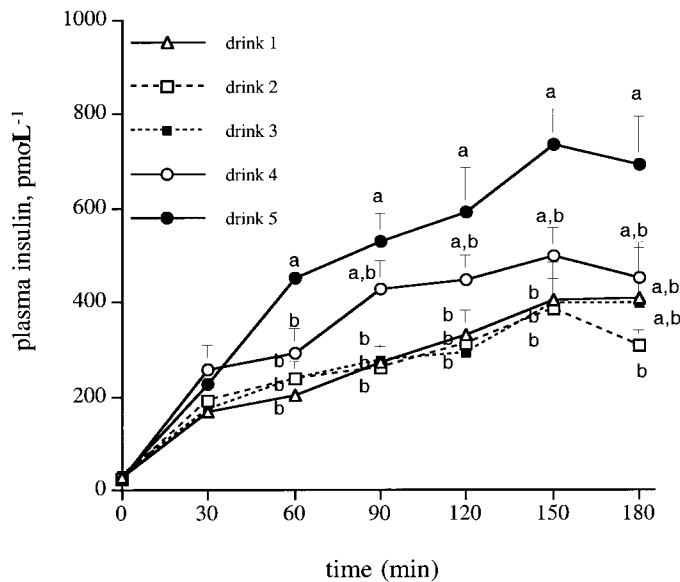
FIGURE 2 Postexercise plasma insulin concentrations after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM ($n = 8$). Mean values not sharing a common superscript are different, $P < 0.05$.

tions and did not cause any gastrointestinal or other complaints.

The aim of the present study was to maximize postexercise insulin levels and to increase plasma amino acid availability in trained athletes. Our data did not show an increase in postexercise insulin response after the ingestion of a wheat protein hydrolysate only (at an intake of 0.2 or 0.4 g · kg^{-1} · h^{-1}) in combination with carbohydrate (1.2 g · kg^{-1} · h^{-1}) compared with the ingestion of only carbohydrate. This is in contrast to earlier findings in the postabsorptive resting state (van Loon et al. 2000a), during which considerable, but nonsignificant, increases in insulin response were observed after the ingestion of carbohydrate (0.8 g · kg^{-1} · h^{-1}) combined with pea, whey or wheat protein hydrolysate in comparison with the ingestion of only carbohydrate. This could be related to the preceding exercise in the present study, because muscle contraction stimulates glucose transport via GLUT4 translocation (Ivy 1997), which is likely to result in a reduction in postexercise insulin response. In addition, endurance trained athletes exhibit a markedly reduced secretory insulin response after glucose administration (Lohmann et al. 1978, Rodnick et al. 1987). However, a significant, additional increase in plasma insulin level occurs after the ingestion of carbohydrate (0.8 g · kg^{-1} · h^{-1}) with a whey or wheat protein hydrolysate (0.4 g · kg^{-1} · h^{-1}) in endurance trained athletes after exercise (van Hall et al. 2000). Therefore, the apparent contradictory findings should be explained by the higher carbohydrate ingestion rate (1.2 versus 0.8 g · kg^{-1} · h^{-1}) that was applied in the present study.

We observed a substantial additional increase in insulin response after the ingestion of the mixtures containing wheat protein hydrolysate in combination with free leucine and phenylalanine. The addition of these free amino acids clearly

led to a significant increase in the insulin response (the area under the curve) compared with the control and wheat protein–only trials (Fig. 3). A dose-effect relationship existed in that doubling the ingestion rate of the hydrolysate–amino acid mixture up to 0.4 g · kg^{-1} · h^{-1} resulted in a substantial increase in insulin response (the area under the curve) compared with the ingestion of only 0.2 g · kg^{-1} · h^{-1} of the same mixture.

Recently, we studied the effects of ingestion of carbohydrate with this mixture of wheat protein hydrolysate, free leucine and phenylalanine on postexercise insulin levels and muscle glycogen synthesis rates in trained athletes (van Loon et al. 2000b). We demonstrated a substantial, additional increase in insulin response after the ingestion of this mixture (0.4 g · kg^{-1} · h^{-1}) in addition to normal postexercise carbohydrate consumption rates (0.8 g · kg^{-1} · h^{-1}). In accordance with Zawadzki et al. (1992), we reported a significant acceleration of muscle glycogen synthesis rates compared with the ingestion of only carbohydrate (0.8 g · kg^{-1} · h^{-1}). However, these increased muscle glycogen synthesis rates were not significantly higher than synthesis rates observed after the ingestion of larger amounts of carbohydrate (1.2 g · kg^{-1} · h^{-1}). Future research will be necessary to investigate whether muscle glycogen synthesis can be further accelerated by ingesting an insulinotropic protein hydrolysate (and amino acid) mixture in combination with a carbohydrate intake of ≥1.2 g kg^{-1} · h^{-1}.

Consistent with recent findings in the postabsorptive resting state (van Loon et al. 2000a), the magnitude of the in vivo insulin response correlated with the increase in plasma leucine, phenylalanine and tyrosine concentrations. Regression analysis showed a strong positive correlation between plasma leucine, phenylalanine and tyrosine concentrations and the insulin response. This suggested relationship is in accordance with the effects of leucine and phenylalanine in vitro in studies with incubated β cells of the pancreas (Blachier et al. 1989a and 1989b, Hutton et al. 1980, Malaisse
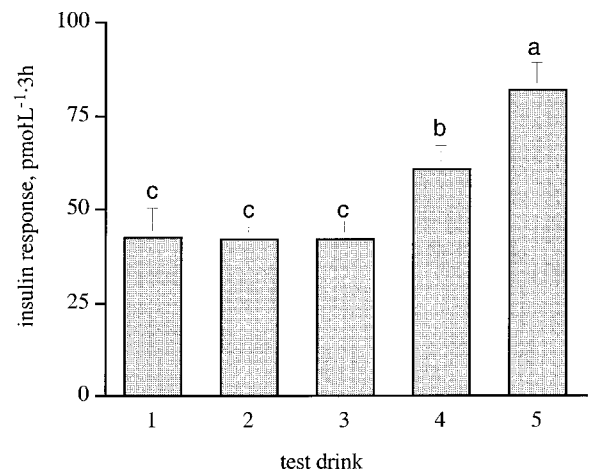
FIGURE 3 Postexercise plasma insulin responses after the ingestion of protein hydrolysate/amino acid–carbohydrate mixtures in humans. Test drink 1, carbohydrate only (1.2 g · kg^{-1} · h^{-1}); drink 2, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}); drink 3, carbohydrate with protein hydrolysate (0.4 g · kg^{-1} · h^{-1}); drink 4, carbohydrate with protein hydrolysate (0.1 g · kg^{-1} · h^{-1}), leucine (0.05 g · kg^{-1} · h^{-1}) and phenylalanine (0.05 g · kg^{-1} · h^{-1}); and drink 5, carbohydrate with protein hydrolysate (0.2 g · kg^{-1} · h^{-1}), leucine (0.1 g · kg^{-1} · h^{-1}) and phenylalanine (0.1 g · kg^{-1} · h^{-1}). Values are means ± SEM ($n = 8$). Mean values not sharing a common superscript are different, $P < 0.05$.

TABLE 2

*Plasma amino acid responses in humans after the ingestion of carbohydrate and protein (hydrolysate)
with or without free amino acids[1]*

	Trials[2]				
Amino acids	Drink 1 control	Drink 2 wheat-0.2	Drink 3 wheat-0.4	Drink 4 wheat/Leu/Phe-0.2	Drink 5 wheat/Leu/Phe-0.4
	$mmol \cdot L^{-1} \cdot 3\ h^{-1}$				
Threonine[3]	−3.36 ± 0.69	−0.32 ± 0.38a	2.06 ± 0.70a	−2.67 ± 0.56c	−2.33 ± 0.34c
Serine	−3.20 ± 0.59	−0.08 ± 0.40a	2.95 ± 0.94a,b	−2.56 ± 0.45c	−1.56 ± 0.24c
Asparagine	−2.22 ± 1.08	−0.21 ± 0.20	0.96 ± 0.48a	−1.67 ± 0.43c	−1.42 ± 0.27
Glutamate	−2.45 ± 1.05	0.97 ± 1.02	−0.01 ± 0.84	−0.33 ± 1.51	−0.56 ± 0.82
Glutamine	−13.93 ± 4.03	−2.85 ± 3.03	6.63 ± 3.72a	−8.42 ± 4.84	−5.03 ± 2.38
Proline	−4.64 ± 1.05	7.82 ± 1.14a	16.86 ± 2.26a,b	−0.92 ± 0.70b,c	2.78 ± 0.50a,c
Glycine	−4.46 ± 1.05	0.29 ± 0.47a	2.99 ± 0.771	−4.07 ± 1.06b,c	−4.49 ± 0.84b,c
Alanine	−11.42 ± 1.76	−4.88 ± 1.19	−1.46 ± 2.55a	−8.66 ± 1.90	−9.58 ± 1.43
Citrulline	−3.07 ± 0.57	−0.73 ± 0.24a	0.30 ± 0.25a	−0.64 ± 0.22a	0.63 ± 0.15a,b
α-Aminobutyrate	−0.55 ± 0.41	0.21 ± 0.10	0.48 ± 0.12a	−0.24 ± 0.11	−0.31 ± 0.06
Valine[3]	−6.10 ± 0.89	0.40 ± 0.64a	6.45 ± 1.19a,b	−9.14 ± 1.05b,c	−6.10 ± 0.71b,c
Methionine[3]	−1.63 ± 0.48	−0.88 ± 0.13	−0.44 ± 0.26	−1.42 ± 0.09	−1.38 ± 0.17
Isoleucine[3]	−3.21 ± 0.56	0.74 ± 0.24a	3.57 ± 0.53a,b	−3.40 ± 0.43b,c	−1.84 ± 0.30b,c
Leucine[3]	−5.30 ± 0.76	1.81 ± 0.46	7.34 ± 0.98a	31.51 ± 1.08a,b,c	66.68 ± 3.29a,b,c,d
Tyrosine[3]	−2.95 ± 0.69	0.20 ± 0.19	1.97 ± 0.53a	3.79 ± 0.47a,b	8.26 ± 1.15a,b,c,d
Phenylalanine[3]	−2.48 ± 0.73	2.17 ± 0.32	4.30 ± 0.47	23.14 ± 2.27a,b,c	53.17 ± 4.14a,b,c,d
Tryptophan[3]	−0.69 ± 0.65	0.74 ± 0.62	1.57 ± 0.55	−1.97 ± 1.96	−0.77 ± 0.54
Ornithine	−1.74 ± 0.54	1.81 ± 0.24a	3.27 ± 0.44a	0.51 ± 0.17a,c	1.86 ± 0.24c
Lysine[3]	−3.82 ± 0.81	−2.03 ± 0.35	−1.64 ± 0.58	−3.15 ± 0.78	−2.98 ± 0.73
Histidine[3]	−2.03 ± 0.60	0.68 ± 0.19a	1.70 ± 0.35a	−1.09 ± 0.22b,c	−0.68 ± 0.30c
Arginine	−4.14 ± 0.80	−1.19 ± 0.44	1.24 ± 0.36a	−1.45 ± 0.58a,c	−0.04 ± 0.68a

[1] Plasma amino acid response expressed as the area under the curve above baseline (means ± SEM; $n = 8$); a,b,c,d significant difference in plasma amino acid response when compared to a specific trial (drink 1, 2, 3, and 4, respectively) ($P < 0.05$).
[2] The applied drinks contain drink 1, carbohydrate only ($1.2\ g \cdot kg^{-1} \cdot h^{-1}$); drink 2, carbohydrate with wheat protein hydrolysate ($0.2\ g \cdot kg^{-1} \cdot h^{-1}$); drink 3, carbohydrate with wheat protein hydrolysate ($0.4\ g \cdot kg^{-1} \cdot h^{-1}$); drink 4, carbohydrate with wheat protein hydrolysate ($0.1\ g \cdot kg^{-1} \cdot h^{-1}$), leucine ($0.05\ g \cdot kg^{-1} \cdot h^{-1}$) and phenylalanine ($0.05\ g \cdot kg^{-1} \cdot h^{-1}$); and drink 5, carbohydrate with wheat protein hydrolysate ($0.2\ g \cdot kg^{-1} \cdot h^{-1}$), leucine ($0.1\ g \cdot kg^{-1} \cdot h^{-1}$) and phenylalanine ($0.1\ g \cdot kg^{-1} \cdot h^{-1}$).
[3] Essential amino acids.

et al. 1991, Malaisse Lagae et al. 1971, Sener et al. 1989 and 1981, Sener and Malaisse, 1980 and 1981, Varnier et al. 1995) and with the in vivo studies by Floyd and coworkers (Fajans et al. 1962, Floyd et al. 1963, 1966, 1968, 1970a and 1970b) in which several (combinations of) amino acids with and without glucose were infused. The positive correlation observed with plasma tyrosine concentrations may be explained by the fact that tyrosine, the hydroxylation product of phenylalanine in the liver, is formed when large amounts of phenylalanine are ingested (Pogson et al. 1985). Furthermore, we observed a less substantial but significant negative correlation between the insulin response and plasma threonine, asparagine, glycine, alanine, valine, methionine, isoleucine and histidine concentrations. These negative correlations could be explained by an increased amino acid uptake after an increase in insulin level. Interestingly, the amino acids that revealed significant negative correlations included all of the essential amino acids (of course, with the exclusion of the supplemented amino acids leucine, phenylalanine and its product tyrosine). Plasma amino acid concentrations were generally lower after the ingestion of drinks 4 and 5 compared with the control trial, although in the latter, considerable amounts of protein and amino acids were ingested, which would normally increase the plasma amino acid response as shown in trials 2 and 3. This seems to suggest that tissue amino acid uptake and possibly also postexercise net muscle protein balance were increased after the ingestion of this insulinotropic mixture. This would be in line with several studies demonstrating that an increase in plasma insulin concentration, during conditions of hyperaminoacidemia, further increases net muscle protein balance in vivo in humans (Fryburg et al. 1995, Gelfand and Barrett, 1987, Hillier et al. 1998). Such a stimulating effect on net protein balance may in part also be a consequence of a stimulating effect of leucine on skeletal muscle protein synthesis, independent of an increase in insulin levels (Anthony et al. 1999 and 2000). However, the potential of insulinotropic protein hydrolysate and amino acid mixtures to stimulate postexercise net muscle protein anabolism, and the mechanisms involved, remains to be investigated.

The present study shows that the ingestion of a mixture of wheat protein hydrolysate, free leucine and phenylalanine, in combination with carbohydrate, results in a substantial, additional increase in the postexercise insulin response compared with the ingestion of only carbohydrate. Furthermore, it is demonstrated that the magnitude of this increase in insulin response is dose dependent. Consequently, this mixture provides a practical tool to strongly elevate postexercise insulin levels via dietary manipulation only. This mixture has previously been shown to stimulate glycogen synthesis after exercise when added to a carbohydrate-containing solution ($0.8\ g \cdot kg^{-1} \cdot h^{-1}$) and may also serve to increase net protein balance in the postexercise phase and be applied as a tool in metabolic research investigating the effects of high plasma insulin concentrations.

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Glycemic, Hormone, and Appetite Responses to Monosaccharide Ingestion in Patients With Type 2 Diabetes

Rosalie Vozzo, Ben Baker, Gary A. Wittert, Judith M. Wishart, Howard Morris, Michael Horowitz, and Ian Chapman

To investigate the relative effects of fructose and glucose on blood glucose, plasma insulin and incretin (glucagon-like peptide-1 [GLP-1] and gastric inhibitory peptide [GIP]) concentrations, and acute food intake, 10 (6 men, 4 women) patients with diet-controlled type 2 diabetes (diabetic) (44 to 71 years) and 10 age and body mass index (BMI)-matched (6 men, 4 women) nondiabetic, control subjects with varying degrees of glucose tolerance (nondiabetic), were studied on 3 days. In random order, they drank equienergetic preloads of glucose (75 g) (GLUC), fructose (75 g) (FRUCT) or vehicle (300 mL water with noncaloric flavoring [VEH]) 3 hours before an ad libitum buffet lunch. Mean glucose concentrations were lower after FRUCT than GLUC in both type 2 diabetics (FRUCT v GLUC: 7.5 ± 0.3 v 10.8 ± 0.4 mmol/L, $P < .001$) and nondiabetics (FRUCT v GLUC: 5.9 ± 0.2 v 7.2 ± 0.3 mmol/L, $P < .05$). Mean insulin concentrations were approximately 50% higher after FRUCT in type 2 diabetics than in nondiabetics (diabetics v nondiabetics: 23.1 ± 0.7 v 15.1 ± 1.3 μU/mL; $P < .0001$). Plasma GLP-1 concentrations after fructose were not different between type 2 diabetics and nondiabetics ($P > .05$). Glucose, but not FRUC, increased GIP concentrations, which were not different between type 2 diabetics and nondiabetics ($P > .05$). Food intake was suppressed 14% by GLUC ($P < .05$ v CONT) and 14% by FRUC ($P < .05$ v CONT), with no difference between the amount of food consumed after GLUC and FRUC treatment in either type 2 diabetics or nondiabetics ($P > .05$). We have confirmed that oral fructose ingestion produces a lower postprandial blood glucose response than equienergetic glucose and demonstrated that (1) fructose produces greater increases in plasma insulin concentration in type 2 diabetics than nondiabetics, not apparently due to greater plasma incretin concentrations and (2) fructose and glucose have equivalent short-term satiating efficiency in both type 2 diabetics and nondiabetics. We conclude that on the basis of improved glycemic control, but not satiating efficiency, fructose may be useful as a replacement for glucose in the diet of obese patients with type 2 diabetes.
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G LUCOSE INGESTION PROMOTES insulin secretion by a direct action on the pancreatic β cells and by stimulating incretin hormone release (glucagon-like peptide-1 [GLP-1] and gastric inhibitory peptide [GIP]). Incretin release accounts for over 50% of the increase in plasma insulin concentrations after ingestion of a glucose load in healthy individuals.[1] Fructose ingestion also induces insulin secretion, but less than that of glucose. While no study has directly compared the effect of fructose ingestion on plasma insulin in adults with and without non–insulin-dependent diabetes mellitus (type 2 diabetes), the results of separate studies[2,3] suggest that oral fructose is a more potent insulin secretagogue in type 2 diabetes. Greater fructose-induced incretin release in people with type 2 diabetes may explain this. Fructose stimulates GLP-1 secretion in nondiabetics, but less than glucose[2] and has no effect on GIP concentrations.[4] In type 2 diabetics, GIP is secreted in response to glucose, but has almost no insulinotropic activity, whereas the extent of GLP-1 release in people with type 2 diabetes after glucose ingestion is unclear; some studies reporting enhanced and some reporting decreased secretion compared with nondiabetics.[5,6] The relative effects of fructose on insulin, GLP-1 amide and GIP release in people with and without type 2 diabetes have not been reported.

The comparative effects of glucose and fructose on appetite remain controversial. Results of several,[7-9] but not all[2,10] studies in healthy people indicate a greater suppression of short-term food intake by oral fructose than equienergetic glucose. The relative satiating effects of these monosaccharides have not been examined in people with diabetes. Intravenous GLP-1 reduces food intake in healthy humans without type 2 diabetes.[11] It is not yet clear if this is a physiologic or pharmacologic effect, but GLP-1 may be an endogenous satiety factor,[12] in which case, its secretion may account for some of the reduction in food intake after glucose and fructose ingestion.

This study was conducted to determine the relative acute effects of oral glucose and fructose on appetite and food intake and plasma insulin and incretin concentrations in people with and without type 2 diabetes.

MATERIALS AND METHODS

Subjects

Ten patients with early (<4 years since diagnosis), well controlled, type 2 diabetes and 10 nondiabetic subjects with varying degrees of impaired glucose tolerance (nondiabetics) were recruited from the Royal Adelaide Hospital diabetes clinic and by advertisement (subject characteristics are detailed in Table 1). The diabetic patients were all treated with diet alone, ie, none was taking oral hypoglycemic agents or insulin. All diabetics met World Health Organization and American Diabetic Association criteria for the diagnosis of diabetes at the time of the study (fasting venous whole blood glucose concentration ≥6.1 mmol/L ($n = 10$) and/or blood glucose concentration ≥10.1 mmol/L 2 hours after 75 g glucose (9 of 10)[13] (diagnostic values for venous whole blood are lower than those for venous plasma or capillary whole blood). All subjects were nonsmokers and unrestrained eaters as determined by a score of less than 10 on the eating restraint questionnaire of Stunkard

Table 1. Characteristics of Subjects and Comparisons Between Nondiabetic and Diabetic Groups

	Nondiabetic	Type 2 Diabetic	P Value
Gender (M/F)	6 M, 4 F	6 M, 4 F	-
Age (yr)	54.7 (44-69)	56.5 (44-71)	.77
Weight (kg)	84.8 (71-120.9)	86.9 (62-112.3)	.64
Body mass index (kg/m²)	30.9 (27-37.7)	30.2 (25.3-36.2)	.67
Duration of known diabetes (mo)	·	18 (0.5-43)	-
Body fat (%)	37.7 (27.4-45.8)	35.4 (20-48.7)	.39
Fasting blood glucose (mmol/L)*	5.6 (5.2-6.0)	7.0 (6.2-8.8)	.001†
HbA$_{1c}$ (%)‡	5.2 (4.9-5.4)	5.9 (5.2-7.1)	.06

NOTE. Body fat was quantified using bioelectrical impedance.[44] Values are mean (range) (except for gender). Comparisons were performed using a Student's unpaired t test.

*Mean of the 3 study days, venous whole blood.

†$P = .001$.

‡HbA$_{1c}$ (glycated hemoglobin) measurements were performed using the HPLC with cation exchange column method of Philcox et al.[45] Reference range for nondiabetics, 4% to 6%.

and Messick.[14] Potential subjects with significant gastrointestinal symptoms, disease or surgery, intake of more than 20 g alcohol/day on a daily basis, and current use of medications that might affect glycemic control, gastrointestinal motor function, or appetite were excluded. The Royal Adelaide Hospital Human Ethics Committee approved the study protocol and written, informed consent was obtained from each subject prior to enrolment.

Protocol

Each subject was studied on 3 occasions, separated by at least 5 days. Subjects maintained their normal diet between study days and refrained from vigorous exercise and alcohol intake for 24 hours before each study. Subjects attended the study center at 8:30 AM following an overnight fast, except for water. On arrival, a blood sampling cannula was inserted into a forearm vein. Subjects remained either seated or lying on a bed during all 3 studies and could, except during the meal, read (but not about food-related topics) or listen to the radio.

Fifteen minutes after intravenous cannulation ($t = 0$ minutes), subjects received, in a random order and single-blind fashion, a noncaloric lemon flavoring (Green's Foods, Glendenning, NSW, Australia) (60 mL) in water (240 mL) (1) alone (vehicle [VEH]), (2) plus glucose (75 g) (GLUC), or (3) plus fructose (75 g) (FRUCT), which was consumed in 2 minutes. Three hours later subjects were offered a buffet meal and asked to eat until comfortably full or 30 minutes had elapsed. Subjects were monitored for 0.5 hours postprandially. Venous blood samples (10 mL) were taken at $t = -15, 0, 5, 10, 15, 30, 45, 60, 75, 90, 120, 150,$ and 180 minutes for measurement of glucose, GLP-1, GIP, and insulin and also at $t = 210$ and 240 minutes for measurement of glucose and insulin. Visual analogue scale questionnaires (VAS)[15] were administered at 15-minute intervals, starting at $t = -15$ minutes except during the meal.

Biochemical Measurements

Blood samples were collected on ice into EDTA tubes containing a protease inhibitor (Trasylol; Bayer, Leverkeusen, Germany). Plasma was obtained by centrifugation at 4°C at 3,200 rpm for 12 minutes, and stored at −20°C until assayed.

Blood glucose. Blood glucose concentration (mmol/L) was measured at the bedside on venous whole blood using a Medisense II glucometer (Medisense, Bedford, MA).[2] The accuracy of this method has been confirmed previously with an R of .961 and slope of 1.005 for the correlation between Medisense II and laboratory measurements on whole venous blood.

Plasma insulin. Plasma insulin concentration was measured using the Abbott IMx Microparticle Enzyme Immunoassay (Abbott Labora-

tories, Diagnostic Division, Dainabot, Tokyo, Japan). The sensitivity of the assay (concentration at 2 SD from the zero standard) was 1.0 μU/mL. The intra-assay coefficients of variation were 4% at 8.3 μU/mL, 2.9% at 40.4 μU/mL, and 2.5% at 121.7 μU/mL. The inter-assay coefficients of variation were 4.5% at 8.3 μU/mL, 3.4% at 40.4 μU/mL, and 3.6% at 121.7 μU/mL.

Plasma GLP-1. Plasma GLP-1 (7-36) concentration was measured after ethanol extraction of plasma samples by a radioimmunoassay method.[2,17] The antibody, provided by Professor S.R. Bloom (Hammersmith Hospital, London, UK), had been raised in a rabbit immunized with GLP-1 (7-36) conjugated to bovine serum albumin (BSA) by carbodiimide. The antibody had 100% cross-reactivity with synthetic entire GLP-1 (7-36), but does not cross-react with GLP-1 (7-37), glucagon, GIP, or other gut or pancreatic peptides and has been demonstrated by chromatography to measure intact GLP-1 (7-36) amide.[16] It is likely that this antibody also reacts with the degraded GLP-1 (9-36) amide. The minimum detectable limit for the assay was approximately 2 pmol/L, and 11 determinations were used to establish an interassay coefficient of variation of 18%.

Plasma GIP. Plasma GIP concentration was measured, after ethanol extraction of plasma samples by a radioimmunoassay method.[18] Commercially available antiserum was used (Peninsula Laboratories, Belmont, CA). Bound from free fragments were separated using the double-antibody technique. The minimum detectable limit for the assay was approximately 15 pmol/L, and the interassay coefficient of variation was 15%.

Assessment of Symptoms

Hunger, fullness, and nausea were assessed using linear VAS[15] in the form of a questionnaire with the opposites of a particular sensation written at either end of a 10-cm horizontal line; for example hungry versus not hungry and full versus empty.[19] Subjects placed a vertical mark at the appropriate place on each line to indicate the strength of that symptom. Sensations associated with appetite were quantified (cm) as a change from baseline, which was the mean of −15, −10, and 0 minute values.

The test meal was a cold buffet lunch of sliced bread (white and whole meal), margarine, mayonnaise, sliced ham, chicken, cheese, tomato, cucumber and lettuce, plain milk, orange juice, tinned fruit-salad, low-fat strawberry yoghurt, chocolate custard, vanilla ice cream, an apple, pear, and banana, all of which were prepared in excess of what the subject would normally be expected to eat.[20] The total energy content of the food offered was approximately 2,400 kcal (10 MJ). All food items were weighed (to the nearest 0.1 g) before and after the meal and the duration of eating recorded (to the nearest minute). Energy
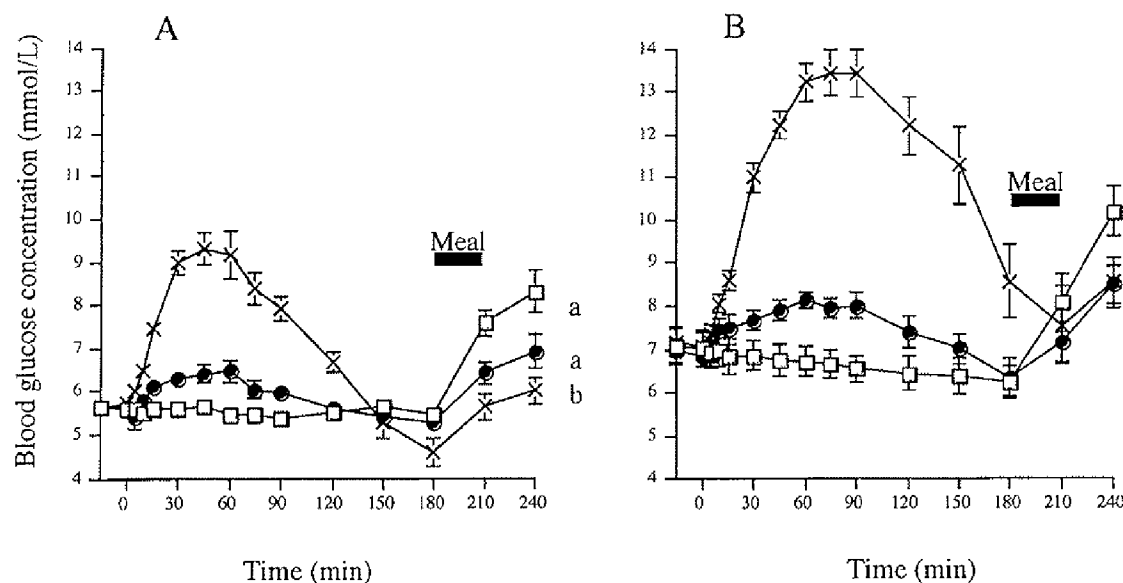
Fig 1. (A) Blood glucose concentrations (mmol/L) after ingestion of glucose (75 g) (GLUC) (x), fructose (75 g) (FRUCT) (●), or vehicle (VEH) ( ) by nondiabetics (n = 10) and (B) by patients with type 2 diabetes (n = 10). Data are mean ± SEM. $P < .001$ for treatment (all treatments were different from each other], $P < .001$ for patient group, $P < .001$ for treatment x patient group ([a,b]different letters indicate that curves are statistically different), $P < .001$ for time by 3-way ANOVA (t = 5 to 180 minutes) with repeated measures followed by contrasts.

consumption (kcal) and macronutrient intake (% of total) were calculated from the amount of food consumed during the buffet meal, using DIET/4 food composition software (Xyris Software, Highgate Hill, Qld, Australia).[20,21]

Statistical Analysis

Baseline data were analysed using 2-way analysis of variance (ANOVA) with repeated measures (treatment x patient group). Hunger, fullness, and nausea, blood glucose and plasma insulin, GLP-1, and GIP concentrations were initially analyzed using a 3-way ANOVA with repeated measures (patient group x treatment x time). When a significant interaction was observed, contrasts were used to test hypotheses of interest, enabling paired comparisons between the studies. Fructose-induced changes in plasma insulin GLP-1 and GIP concentrations were assessed using 2-way ANOVA with repeated measures (patient group x time). Relationships between baseline and fructose-induced increases in blood glucose and plasma insulin concentrations were assessed using Pearson's correlations. Food intake was compared using a 2-way ANOVA (treatment x patient group) with repeated measures. Subject characteristics were compared with Student's unpaired t tests. SuperANOVA Version 1.11 (Abacus Concepts, Berkley, CA) software was used to perform these analyses. A P value of less than .05 was considered statistically significant. All data are expressed as means ± SEM.

RESULTS

One subject experienced a severe headache prior to commencing the buffet meal on the fructose treatment day; all food intake data for this subject were omitted from the food intake analysis. All other subjects tolerated studies well; with no untoward side effects reported.

Biochemical Measurements

Blood glucose. On the basis of their responses to the 75-g oral glucose load, 7 of the nondiabetic subjects had impaired glucose tolerance, 2 had impaired fasting, and 1 normal glucose tolerance (Fig 1).

Fasting (baseline) blood glucose concentrations (mean of −15 and 0 minutes) were greater in type 2 diabetics than in nondiabetics (7.0 ± 0.2 v 5.6 ± 0.1 mmol/L, $P < .001$) with no difference between the 3 treatment days for either subject group ($P > .05$).

Blood glucose concentrations were higher in type 2 diabetics than in nondiabetics after both GLUC (mean, 5 to 180 minutes) (10.8 ± 0.4 v 7.2 ± 0.3 mmol/L; $P < .001$) and FRUCT (mean, 7.5 ± 0.3 v 5.9 ± 0.2 mmol/L; $P < .001$). The mean blood glucose concentration after GLUC, but not FRUCT, was greater than after VEH in all subjects ($P < .001$). At the start of the meal ingestion, blood glucose concentrations were higher in type 2 diabetics than in nondiabetics (7.1 ± 0.7 v 5.1 ± 0.2 mmol/L, $P < .01$) and were higher after GLUC than FRUCT in type 2 diabetics (8.6 ± 0.9 v 6.4 ± 0.4 mmol/L; $P < .05$). Blood glucose concentrations after lunch (mean of t = 210 and 240 minutes) were lower after GLUC ($P < .001$) and FRUCT ($P < .001$) than VEH, with no difference between the monosaccharides (type 2 diabetics, GLUC v FRUCT: 8.1 ± 0.8 v 7.8 ± 0.5 mmol/L, $P > .05$, nondiabetics; GLUC v FRUCT: 5.8 ± 0.2 v 6.6 ± 0.2 mmol/L, $P > .05$) in all subjects.

Plasma insulin. Baseline plasma insulin concentrations were similar in type 2 diabetics and nondiabetics (12.2 ± 0.9 v
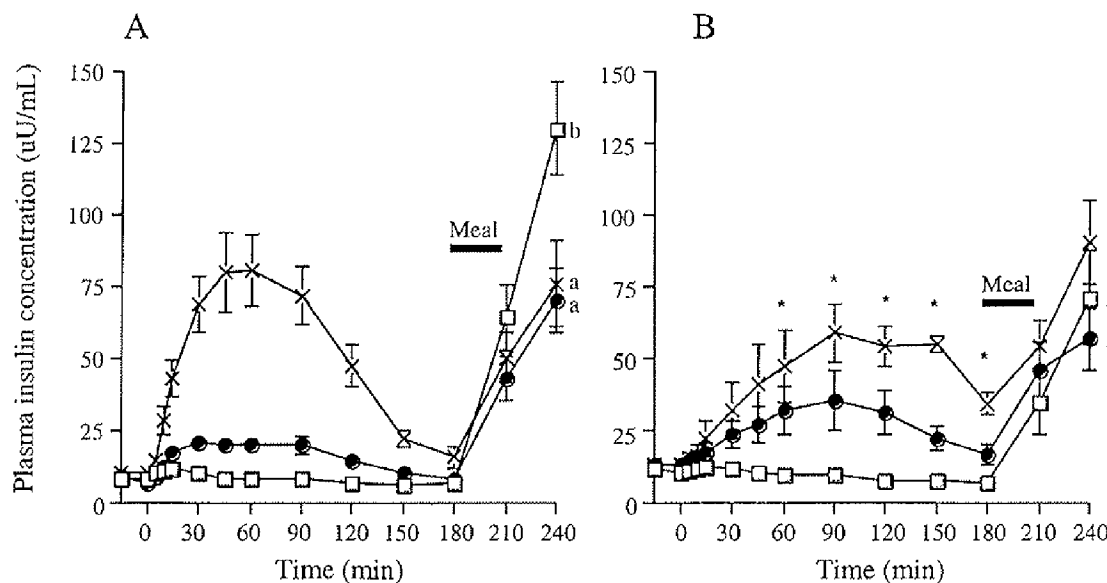
A

B

Fig 2. (A) Plasma insulin concentrations (μU/mL) after ingestion of glucose (75 g) (GLUC) (✕), fructose (75 g) (FRUCT) (●), or vehicle (VEH) (▽) by nondiabetics (n = 10) and (B) by patients with type 2 diabetes (n = 10). Data are mean ± SEM. $P < .001$ for treatment (all treatments were different from each other), $P < .05$ for treatment × patient group ([a,b]indicates that curves are not statistically different), $P < .001$ for time by 3 way ANOVA ($t = 5$ to 180 minutes) with repeated measures followed by contrasts. $P < .001$ for time × patient group (* indicates time points at which fructose-induced plasma insulin concentrations in type 2 diabetics differ from nondiabetics), $P < .001$ for time by 2-way ANOVA ($t = 5$ to 180 minutes) with repeated measures.

8.6 ± 0.6 μU/mL; $P = .2$) with no differences between the treatment days in either subject group (Fig 2). Mean plasma insulin concentrations were significantly ($P < .01$) higher ($t = 5$ to 180 minutes) after GLUC than after both FRUCT and VEH in type 2 diabetics and nondiabetics. Overall, insulin concentrations were higher after FRUCT than VEH ($P < .001$), but were significantly higher only in type 2 diabetics ($P < .01$). From 60 to 180 minutes after fructose ingestion, plasma insulin concentrations were significantly higher in subjects with type 2 diabetes than in nondiabetics (time × group, $P < .001$) and mean insulin concentrations after fructose ($t = 60$ to 180 minutes) were approximately 50% higher in type 2 diabetics (23.1 ± 0.7 v 15.1 ± 1.3 μU/mL; $P < .05$). Fructose-induced increases in plasma insulin concentrations from baseline were also greater in type 2 diabetics than in nondiabetics ($P < .05$ time × patient group). The time to peak plasma insulin concentrations was delayed in type 2 diabetics compared with in nondiabetics, regardless of the monosaccharide ingested (74.2 ± 8.7 v 51.5 ± 8.0 minutes; $P < .01$). After fructose ingestion, plasma insulin concentrations remained higher in type 2 diabetics than in nondiabetics at the start of the buffet meal (16.4 ± 3.3 v 8.0 ± 0.8 μU/mL, $P < .05$). Postbuffet meal (mean $t = 210$ and 240 minutes) plasma insulin concentrations on the fructose day did not differ between type 2 diabetics and nondiabetics (50.1 ± 10.4 v 56.3 ± 9.4 μU/mL, $P > .05$).

Plasma insulin concentrations (5 to 180 minutes) after FRUCT ingestion were not significantly correlated with baseline blood glucose concentrations in the whole subject group

($r = -.1$, $P > .05$) or in either nondiabetics ($r = -.48$, $P > .05$) or type 2 diabetics ($r = -.38$, $P > .05$) when they were assessed separately. Similarly, increases in plasma insulin concentration (mean (5 to 180 minutes) concentration - baseline concentration) after fructose ingestion were not significantly correlated with increases in blood glucose after fructose ingestion in the whole group ($r = -.19$, $P > .05$), type 2 diabetics ($r = .3$, $P > .05$) or in nondiabetics ($r = .6$, $P = .07$).

Plasma GLP-1. Baseline plasma GLP-1 concentrations were slightly, but not significantly, higher in type 2 diabetics than nondiabetics (10.6 ± 1.9 v 8.0 ± 1.3 pmol/L, $P = .12$), with no difference between 3 treatment days in either subject group ($P > .05$) (Fig 3). Overall, GLP-1 concentrations (mean 5 to 180 minutes) were higher after both GLUC and FRUCT than VEH ($P < .001$), but not different after GLUC compared with FRUCT ($P > .05$). Plasma GLP-1 concentrations after fructose were not different between type 2 diabetics and nondiabetics (14.0 ± 0.1 v 10.2 ± 0.1 pmol/L; $P > .05$). GLP-1 concentrations were not different after GLUC than FRUCT in nondiabetics (12.2 ± 0.5 v 10.2 ± 0.5 pmol/L; $P > .05$) or in type 2 diabetics (13.9 ± 0.1 v 14.1 ± 0.6 pmol/L; $P > .05$). At the start of the meal, plasma GLP-1 concentrations were similar in type 2 diabetics and nondiabetics (10.8 ± 1.7 v 11.4 ± 1.4 pmol/L, $P > .05$) and not different after different treatments in either subject group ($P > .05$).

Plasma GIP. Baseline GIP concentrations were similar in type 2 diabetics and nondiabetics (40.9 ± 3.1 v 32.6 ± 6.8 pmol/L, $P = .4$), with no difference between the treatments in
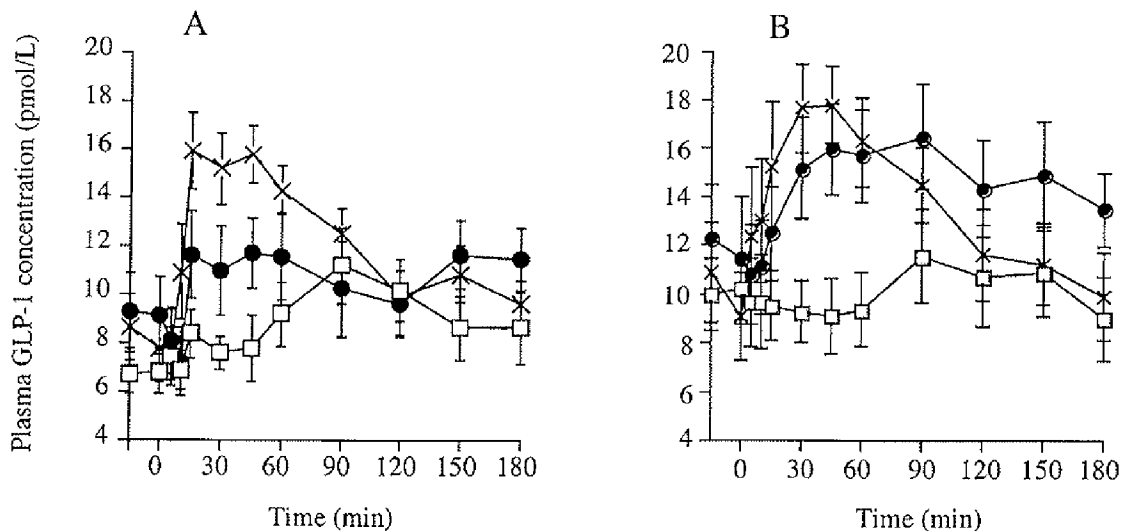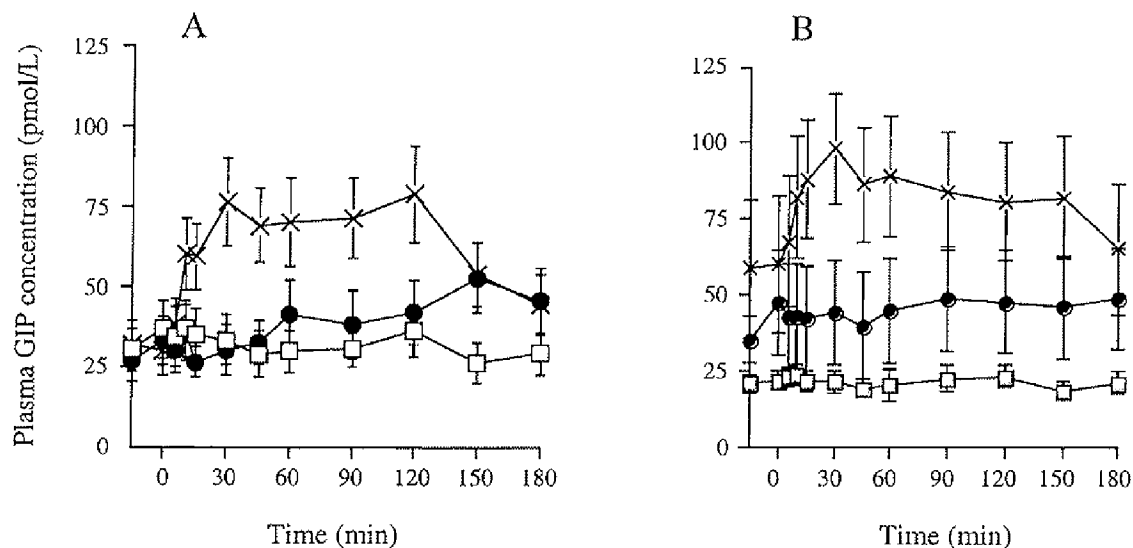
Fig 3. (A) GLP-1 concentration (pmol/L) after ingestion of glucose (75 g) (GLUC) (✖), fructose (75 g) (FRUCT) (●), or vehicle (VEH) (□) by nondiabetics (n = 10) and (B) by patients with type 2 diabetes (n = 10). Data are mean ± SEM. P < .001 for treatment (VEH v GLUC, VEH v FRUCT), P < .001 for time by 3-way ANOVA (t = 5 to 180 minutes) with repeated measures followed by contrasts.

either subject group (P > .05) (Fig 4). Plasma GIP concentrations increased following GLUC, but not after FRUC or VEH in the type 2 diabetics or nondiabetics, so GIP concentrations were significantly higher after GLUC than both FRUC and VEH, with no difference between FRUC and VEH (P > .05). Plasma GIP concentrations after FRUCT were similar in type 2 diabetics and nondiabetics (P > .05).

Appetite and Food Intake

Sensations of appetite. Baseline hunger, fullness, and nausea (P > .05) did not differ between the 3 treatment days for either type 2 diabetics or nondiabetics (Fig 5).

Both monosaccharides increased fullness (GLUC v VEH, P < .01; FRUCT v VEH, P < .05) and decreased hunger

Fig 4. (A) Plasma GIP concentrations (pmol/L) after ingestion of glucose (75 g) (GLUC) (✖), fructose (75 g) (FRUCT) (●), or vehicle (VEH) (□) by nondiabetics (n = 10) and (B) by patients with type 2 diabetes (n = 10). Data are mean ± SEM. P < .001 for treatment (VEH v GLUC, FRUCT v GLUC) and P < .001 for time by 3-way ANOVA (t = 5 to 180 minutes) with repeated measures followed by contrasts.
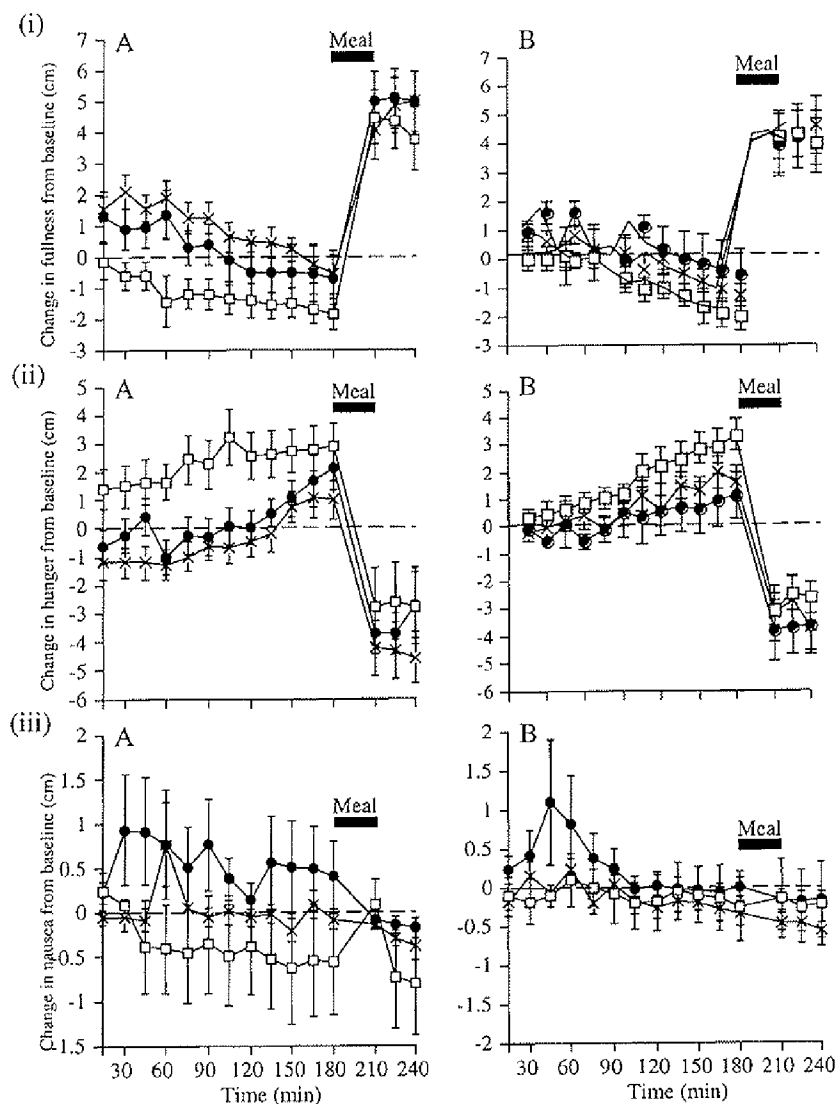
Fig 5. The effect of oral glucose (75 g) (GLUC) (✗), fructose (75 g) (FRUCT) (●), or vehicle (VEH) (▢) on sensations of (i) fullness, (ii) hunger, and (iii) nausea in (A) nondiabetics (n = 10) and (B) in patients with type 2 diabetes (n = 10). Data are mean ± SEM. For sensations of hunger and fullness, $P < .001$ for treatment (VEH v GLUC, VEH v FRUCT) and $P < .001$ for time, and for the sensation of nausea, $P < .001$ for time, by 3-way ANOVA (t = 5 to 180 minutes) with repeated measures followed by contrasts.

(GLUC v VEH and FRUCT v VEH, $P < .05$), and neither had an effect on nausea ($P > .05$). Nausea was higher after FRUCT than GLUC and VEH, more so in nondiabetics than type 2 diabetic subjects, although this difference was not statistically significant ($P = .07$). There were no differences in hunger or fullness perceptions between GLUC and FRUCT in either subject group ($P > .05$ for type 2 diabetics and nondiabetics).

Food intake. No subject consumed all the food offered, but 1 subject ate for the full 30 minutes on all of the 3 treatment days. Energy intake was suppressed approximately 14% (~147 kcal) compared with VEH, by 75 g monosaccharide ingestion ($P < .05$), with no difference between the suppressive effect of GLUC and FRUCT (908 ± 97 kcal, ~14% suppression v 901 ± 71 kcal, ~14% suppression, $P > .05$). The suppression

of food intake after monosaccharide ingestion represents approximately 40% of the energy content of the preloads. Diabetics ate less than nondiabetics on every study day (Fig 6) and about 23% less overall (type 2 diabetics v nondiabetics: 824 ± 69 v 1,070 ± 71 kcal; $P > .05$). There was no patient group × treatment interaction ($P > .05$) and no differences in the macronutrient composition of the foods eaten on different days ($P > .05$) or eaten by type 2 diabetics compared with nondiabetics ($P > .05$).

DISCUSSION

We have confirmed that fructose ingestion produces smaller increases in blood glucose concentrations than glucose inges-
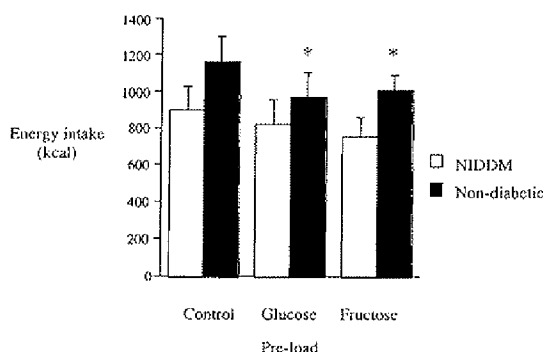
Fig 6. Food intake at a buffet meal 3 hours after ingestion of glucose (75 g) (GLUC), fructose (75 g) (FRUCT), or vehicle (VEH) by nondiabetics (solid bars) (n = 10) and by patients with type 2 diabetes (open bars) (n = 9). Data are mean ± SEM. *Indicates a significant effect of preload $P < .05$ (VEH v GLUC, VEH v FRUCT) by 2-way ANOVA with repeated measures.

tion in subjects with and without type 2 diabetes. In addition, we provide evidence that fructose ingestion is associated with higher plasma insulin concentrations in diabetics than in nondiabetics and without greater increases in diabetics of plasma GLP-1 or GIP concentrations. Consistent with our previous finding that oral fructose and glucose were equally satiating in lean, young adults without diabetes,[2] we have now found them to be equally satiating in older, overweight people with and without type 2 diabetes.

Our finding of lower blood glucose concentrations after fructose than glucose ingestion is consistent with the results of previous studies in people with diabetes[3,22] and obese[23] and nonobese[2] people without diabetes, showing smaller increases in blood glucose concentration after ingestion of fructose than glucose or the glucose-containing disaccharides, sucrose and lactose.[24] The small increase in circulating glucose concentrations after fructose ingestion is probably the result of hepatic conversion of fructose to glucose.[25,26] Lowering blood glucose concentrations in people with diabetes reduces the microvascular and probably macrovascular complications of this disease.[27,28] Therefore, substitution of fructose for glucose in the diet of diabetics may represent a way of lowering average blood glucose concentrations and possibly reducing hyperglycemia-induced diabetic complication responses. The present study evaluates responses to a supraphysiologic dose (75 g) of fructose. Several studies that have examined the chronic effect of including smaller doses of fructose in the diet of diabetics, either as a supplement,[29-32] or instead of other sugars,[33] demonstrate either no change or improved glycemic control after 3 to 6 months. There are, however, suggestions that chronic fructose ingestion may increase circulating triglyceride and/or cholesterol concentrations.[34] While addition of fructose to the diabetic diet for its glucose-lowering effects is a practical treatment option, it cannot be assumed that this will reduce metabolic complications. Studies to determine if this is so have not been performed.

We have demonstrated for the first time in this study that

plasma insulin concentrations are higher after fructose ingestion in subjects with type 2 diabetes than in nondiabetic subjects. Although insulin secretion was not directly assessed, this finding suggests that fructose is a more potent insulin secretagogue in type 2 diabetics than in nondiabetics. We compared overweight people with type 2 diabetes who had good glycemic control on diet alone to age- and weight-matched nondiabetic controls. It is not surprising to find a significant degree of impaired glucose tolerance in an older and overweight nondiabetic healthy population. This highlights the contribution of increasing body weight to the development of insulin resistance, impaired glucose tolerance, and eventually diabetes mellitus. It is likely that the differences between diabetics and nondiabetics would have been even more marked if we compared a lean, nondiabetic population with a group of diabetics with worse glycemic control than those in this study.

We hypothesized that a greater increase in plasma insulin concentrations in response to fructose in diabetics than nondiabetics would be due to a greater release of 1 or more of the incretin hormones, GLP-1 and GIP. We found no evidence for this. Although absolute plasma GLP-1 concentrations were (nonsignificantly) higher after fructose in diabetics than in nondiabetics, this was probably due to higher basal GLP-1 concentrations in diabetics. As previously reported,[35] the increase in plasma GLP-1 concentrations after fructose was similar in the 2 subject groups (from ~8 to 12 pmol/L in the nondiabetics and from ~11 to 16 pmol/L in diabetics). As our assay measures both active and inactive GLP-1, we do not know the exact proportions of each and cannot exclude the possibility that the ratio of active:inactive GLP-1 is different in diabetics to nondiabetics. Similarly, we cannot be sure that the degradation of GLP-1 after monosaccharide ingestion will be similar after glucose and fructose. There was a greater variability in plasma GLP-1 concentrations in the diabetics than nondiabetics, and our sample size was relatively small, so a type 2 statistical error cannot be excluded. Nonetheless, the finding of similar plasma GLP-1 concentrations after fructose ingestion in people with and without type 2 diabetes is consistent with a recent report by Toft et al[35] that plasma GLP-1 concentrations after a mixed meal are similar in type 2 diabetics and nondiabetics. While increased GLP-1 release seems unlikely to be a cause of the greater plasma insulin concentrations in diabetics, increased sensitivity to the insulinotropic actions of GLP-1 remains a possibility. Baseline GIP concentrations were higher (although not significantly) in diabetics, possibly as a consequence of the glucose-dependent nature of GIP release.[36] As previously reported,[37] there was no increase in plasma GIP concentration after fructose ingestion in either type 2 diabetics or nondiabetics.

Fructose-induced insulin release is known to be glucose-dependent and may be enhanced in type 2 diabetics by the hyperglycemia characterizing this condition. In vitro pancreas and isolated islet preparation studies show that fructose is incapable of stimulating insulin in the complete absence of glucose,[38-40] and insulin release after intravenous fructose is greater during hyper- than during euglycemia in nondiabetics.[41] Fructose has only a weak insulinogenic action during euglycemia in people without diabetes, but elevation of the blood glucose concentration even slightly (eg, from 5.5 to 6.4

mmol/L) substantially increases the stimulatory effect of fructose on insulin release.[23] Mean baseline blood glucose levels were similarly elevated in the diabetic compared with the nondiabetic subjects in this study (7.0 v 5.6 mmol/L). In addition to enhancing the stimulatory effects of fructose on the β cell, hyperglycemia may indirectly enhance the insulinotropic effects of fructose by increasing the insulinotropic actions of GLP-1. GLP-1 acts on the pancreatic β cell to stimulate insulin secretion, and this insulinotropic effect is enhanced by hyperglycemia.[42] Although hyperglycemia may provide an explanation for the greater fructose-induced increases in plasma insulin concentrations in diabetics, in the present study, the lack of a significant correlation between the increase in plasma insulin concentration after fructose ingestion and either fasting blood glucose concentration or the increase in glucose concentrations after ingestion of the preload does not support this. Nevertheless, the substantial variances in insulin concentrations in the diabetic subjects and the relatively small subject numbers mean that this possibility cannot be excluded, and further studies will be needed to explore this possibility.

The relative effects of monosaccharides on food intake are controversial. Three previous studies, all by Rodin et al,[7,9,43] have found oral fructose ingestion to be more satiating than isoenergetic glucose in subjects without diabetes, whereas 3 other studies, including this one, show no difference.[2,10] The discrepancy may be related to differences in study design. Studies in which fructose has been more satiating than glucose have tended to use higher volume preloads (500 mL v 300 mL in our study[7]) and have shorter time periods between preload and test meal ingestion. We have also used higher concentrations of sugar in the preload than did Rodin et al (75 g in 300 mL v 50 g in 500 mL). This may result in small intestinal

satiety receptors becoming saturated with the monosaccharide, thus producing similar satiating effects of the 2 sugars.

The dose of fructose used in this study was supraphysiologic. It is conceivable that fructose may have suppressed appetite and food intake, in part, by producing a side effect such as nausea. This seems unlikely. The fructose solution in this study was sweeter than the glucose solution, and nausea ratings were somewhat higher in the first 60 minutes after fructose ingestion than glucose ingestion in both diabetics and nondiabetics. Nevertheless, this difference was not significant, and any nauseating effect of fructose, as indicated by these ratings, had resolved by the time of meal ingestion. No subject spontaneously complained of any side effects from fructose ingestion.

This was an acute study that involved the ingestion of each monosaccharide in isolation. It did not investigate the possibility that fructose and glucose have different effects on satiety or blood glucose when ingested chronically from naturally occurring sources (eg, fructose in fruit). Nevertheless, our findings do not support a use for fructose in the diet of people with type 2 diabetes as a means of suppressing food intake and reducing body weight. Furthermore, we studied diabetic patients soon after diagnosis (all <4 years) on diet treatment alone. We cannot be sure that fructose will have the same effect in patients who have had diabetes for longer and/or those who are taking hypoglycemic medication. Further studies will be needed to examine the reduced glycemic response to dietary fructose, particularly its effects on long-term diabetic complications.

In summary, we have found that in people with and without type 2 diabetes, oral fructose ingestion produces a smaller increase in blood glucose concentration than equienergetic oral glucose ingestion, a greater increase in plasma insulin concentration in diabetics than in nondiabetics, and is as satiating as glucose.

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The effect of chain length on glucose absorption and the related metabolic response[1]

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ABSTRACT   The relative absorption of saccharides of different chain length has been reexamined. Whether glucose is administered as a monosaccharide, a disaccharide (maltose), an intermediate polysaccharide mixture with a mean chain length of five glucose units (Caloreen), or a polysaccharide (starch), the rate of rise and fall in blood glucose concentration is similar in healthy subjects. In a maturity onset diabetic, peak blood glucose is similar whether monosaccharide or Caloreen is ingested. Plasma insulin and plasma free fatty acid responses to glucose saccharide ingestion do not appear to be affected by chain length. The dietary form in which starch is administered, rather than chain length, is probably important.   *Am. J. Clin. Nutr.* 31: 1998–2001, 1978

It is now known that hydrolysis of the disaccharide maltose, an oligosaccharide product of digestion, and sucrose proceeds rapidly at the small intestinal brush border and is not rate limiting (1). Nevertheless, a view that arose about 1920 (2) that the monosaccharide, glucose, is absorbed more quickly than starch, a glucose polysaccharide, persists in the literature (3, 4). There is already some evidence that, if this is so, it is not dependent on chain length (5–11). We have sought to clarify the role of saccharide chain length in glucose absorption by examining subjects following the ingestion of mono-, di-, "penta-", and polysaccharides consisting exclusively of glucose.

Materials and methods

Six healthy male volunteers ages 18 to 21 years and one male maturity onset diabetic age 68 were studied. Healthy subjects were divided into two groups. Group I was studied after the ingestion of glucose, Caloreen or starch (cornflour with a composition of 0.25 g protein for every 50 g carbohydrate). Caloreen is a commercially available glucose saccharide mixture that contains 3% glucose, 7% maltose, 5% maltotriose, and 85% polysaccharides of four to 15 (mean five) glucose units (12); it is referred to in this work as a "pentasaccharide". Group II was studied after the ingestion of glucose or maltose (Ajax Chemicals), a glucose disaccharide.

Three days before the first study and between each study, each subject was maintained on a 300-g carbohydrate-enriched diet in order to maximize and standardize glucose tolerance (13, 14). Studies were separated from each other by 3 to 7 days. The order of studies was randomized. The amount of carbohydrate administered was 50 g in each case. All carbohydrates were flavored with 2.5 ml vanilla essence because this made the cornflour starch more palatable. The volume of fluid accompanying each study was adjusted to 500 ml.

Before the series in which a subject participated, he assessed the rate at which he could comfortably ingest the cornflour starch preparation and the time taken was then applied to each saccharide when tested. The maximum time for ingestion was 5 min.

Whole blood glucose was measured by an automated glucose oxidase method (15); plasma insulin immunoreactivity measurements were based on the double antibody method of Hales and Randle (16); plasma free fatty acids (FFA) were assayed by the method of Trout et al. (17).

The significance of difference between events at given times after the ingestion of different saccharides were assessed by analysis of variance and also by the paired *t* test (18). Areas under curves were calculated by the trapezoidal rule (19).

Results

For healthy subjects, the blood glucose concentration after ingestion of different glucose polymers is shown in Figure 1. In group I subjects, there was no significant difference between the time course of absorption of mono-, penta-, or polysaccharide. This is confirmed by an analysis of the area under the curves at hourly intervals (Table 1). With group II subjects, there were no apparent differences between the blood glucose concentrations after the ingestion of monosaccharide and disaccharide.

The patient with maturity onset diabetes also demonstrated a similar time course for blood glucose concentration after the ingestion of mono- and pentasaccharide (Fig. 2).

The plasma insulin responses to mono-, penta-, and poly-saccharide ingestion in group I subjects were not significantly different (Fig. 3; Table 2). In group II subjects, disaccharide ingestion did not induce an insulin response different from that seen with monosaccharide.

Plasma FFA were lowered maximally by 90 min and the overall FFA response found to be almost identical after ingestion of mono-, penta-, and polysaccharides (Fig. 4). Similarly, the FFA response to disaccharide was almost identical with that to monosaccharides.

Discussion

The present study allows the conclusion that chain length alone does not influence the rate of absorption of glucose significantly. Although Caloreen is not an oligosaccharide of uniform chain length, it is a mixture approximating a pentasaccharide. The small
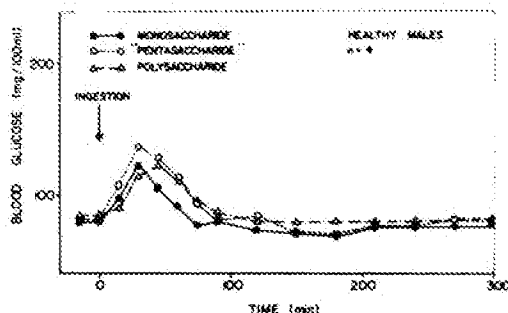

FIG. 1. Blood glucose concentrations in healthy males after ingestion of a monosaccharide (glucose), a pentasaccharide (Caloreen), or a polysaccharide (starch) in four individuals.
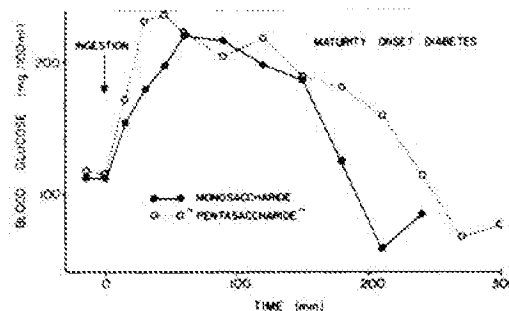

FIG. 2. Blood glucose concentrations in a maturity onset diabetic after ingestion of a monosaccharide (glucose) or a pentasaccharide (Caloreen).

TABLE 1
Area under blood glucose curve after ingestion of glucose polymers
of different chain lengths[a]

Subject group	Saccharide[c]	Hours after ingestion[b]					
		1	2	3	4	5	Total
I	Mono	6184	4388	4223	4392	4583	23768
		±592	±973	±289	±292	±241	±2251
$n = 4$	Penta	6925[d]	5243[d]	4474[d]	4496[d]	4871[d]	26009[d]
		±330	±561	±222	± 84	±102	± 706
	Poly	6369[d]	5434[d]	4800[d]	4815[d]	4890[d]	26308[d]
		±319	±750	± 99	± 52	± 50	±1119
II	Mono	7590	6218	5100	5213	5273	29393
$n = 2$	Di	7306	4163	4710	4710	4980	25869

[a] Area was calculated with time as the abscissa in minutes and blood glucose concentration as the ordinate in mg/100 ml. [b] Mean ± SEM of the area is given for each hour after the ingestion of saccharide and for the total duration of the test period. [c] Significance of difference from monosaccharide in a given time period is indicated by ns $P > 0.05$. [d] Not significant.

proportion (3%) of glucose monomer present would not account for the similarity of monosaccharide and Caloreen blood glucose responses. If chain length were rate limiting in glucose absorption, starch should produce a more prolonged blood glucose response than Caloreen and this, in turn, a more prolonged response than the monosaccharide glucose. If anything, there was a tendency for the oligosaccharide mixture, Caloreen, to produce a slightly larger glucose rise and insulin response than either mono- or polysaccharide. However, any differences in glucose response are unlikely to be of any biological or practical consequence. For example, the recommendation of glucose over starch as such to produce a more rapid rise in blood glucose would appear to be ill-founded.

The present investigation answers the question of the effect of chain length on glucose absorption more directly than previous investigations that have examined saccharides of different carbohydrate composition (10, 11), not considered intermediate chain length saccharides (5-8, 11) or included saccharides in test meals where factors other than chain length could be important (5, 10). The similarity of plasma insulin and FFA responses to glucose absorption is further confirmation of the lack of importance of chain length in glucose absorption not available in other studies (5-9).

It is known that the ingestion of the disaccharide sucrose leads to lower blood glucose concentration than does monosaccharide ingestion (20), but this may reflect the different mode of assimilation of the fructose part of sucrose rather than chain length. Also, the disaccharide lactose, with its glucose and galactose components, is subject to rate limiting hydrolysis in the small intestine (1) and consequently would not necessarily produce
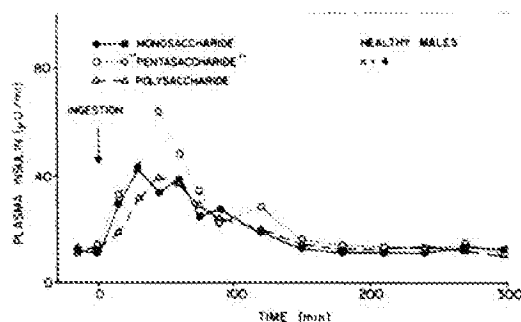

FIG. 3. Plasma insulin immunoreactivities in healthy males after ingestion of various glucose polymers. See legend to Figure 1.
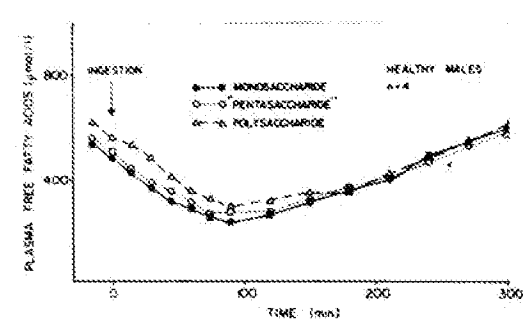

FIG. 4. Plasma free fatty acid concentrations in healthy males after ingestion of various glucose polymers. See legend to Figure 1.

TABLE 2
Area under plasma insulin curve after ingestion of glucose polymers of different chain lengths[a]

Subject	Saccharide	Hours after ingestion[b]					
		1	2	3	4	5	Total
I	Mono	1719	1562	858	685	672	5495
		±254	±469	±223	±140	±114	±1073
n = 4	Penta	2495[c]	1809[c]	1168[c]	753[c]	814[c]	7038[c]
		±496	±696	±343	±113	±167	±1674
	Poly	1715[c]	1513[c]	969[c]	742[c]	684[c]	5621[c]
		±234	±469	±217	±163	±136	±1169
II	Mono	2866	1635	1019	871	865	7256
n = 2	Di	2945	1581	890	715	773	6903

[a] Area was calculated with time as the abscissa in minutes and plasma insulin immunoreactivity as the ordinate in microunits per milliliter. [b] See footnotes to Table 1. [c] Not significant.

comparable rises in blood sugar concentration by comparison with monosaccharide.

Our studies do not exclude the possibility that dietary accompaniments may modify glucose absorption when chain length is comparable. There is an increasing literature on this subject, largely with respect to dietary fiber content (21). It has also been suggested that cooking renders starch more readily absorbable (22).

Our deductions about the lack of importance of chain length may also need to be qualified in the context of exocrine pancreatic deficiency, as in the first few weeks of life (23) and in exocrine pancreatic disease (8), where α-amylase is deficient.

The similarity of blood glucose concentrations after glucose saccharide and maltose ingestion is of additional interest inasmuch as maltose can be absorbed as such and metabolized after intravenous administration (24, 25). Nevertheless, maltose is usually hydrolyzed more rapidly than it can be absorbed (26). This, presumably, has been the case in the present study to allow blood glucose concentrations comparable to those after the same amount of ingested glucose monomer.

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Metabolic response to glucose ingested with various amounts of protein[1-3]

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ABSTRACT   Seven healthy, normal-weight subjects were fed breakfasts of 50 g protein, 50 g glucose, and 10, 30, or 50 g protein plus 50 g glucose in random sequence. Plasma glucose, insulin, C peptide, glucagon, nonesterified fatty acids, and α-amino nitrogen were then measured from samples obtained over 4 h. The postmeal net area of each response curve was calculated. Ingestion of 50 g protein alone did not change the serum glucose concentration. The various amounts of protein ingested with 50 g glucose also did not alter the serum glucose response compared with that observed with 50 g glucose alone. Ingestion of the various amounts of protein also did not result in a further increase in insulin concentration when ingested with glucose, except with the 50-g-protein dose. This increase was modest. Ingestion of glucose resulted in a decrease in α-amino nitrogen and glucagon concentrations whereas ingestion of protein increased them as expected. Additions of progressively larger amounts of protein to the glucose meal resulted in a progressive increase in the α-amino-nitrogen- and glucagon-area responses. The relationship was curvilinear for both the α-amino-nitrogen response and the glucagon response. The null point, that is, the protein dose ingested with 50 g glucose at which there would be no change in area response, was estimated to be 9 g protein for α-amino nitrogen and 5 g protein for glucagon.   *Am J Clin Nutr* 1990;52: 267–72.

KEY WORDS   Dietary protein, diet, insulin, C peptide, α-amino nitrogen, glucagon, glycemic index, relative glucose area

Introduction

It has been well established that both protein and amino acid ingestion stimulate insulin secretion and thus may affect the postprandial glucose concentration (1–6). However, few studies have been published that have quantitated metabolic responses on the basis of differing amounts of protein in the meal. We (7) previously demonstrated in subjects with non-insulin-dependent diabetes (NIDDM), fed meals of 50 g glucose with various amounts of protein, an insulin incremental area response that was essentially linear with respect to the quantity of protein ingested. The increasing insulin response was associated with a decreasing plasma glucose response.

Having demonstrated a sensitive relationship between the doses of protein and the insulin response in subjects with NIDDM, we were interested in determining the sensitivity of the metabolic response of normal subjects to differing doses of protein. Therefore, normal subjects were studied by use of the same protocol. We (8) previously published data on the metabolic response of normal individuals to 50 g glucose, 50 g protein, and both together. We report here data on the response of serum glucose and insulin to various doses of protein given with 50 g glucose in these same subjects.

Methods

Four normal males and three normal females were studied in our metabolic unit. Their mean ($\pm$SD) age was 35.6 $\pm$ 7.9 y (range 27–54 y). All were within 5% of desirable body weight according to the 1959 Metropolitan Life Insurance Company tables (9). All subjects gave written, informed consent, and the study was approved by the Medical Center's Committee on Human Subjects. All participants had ingested a diet containing $\geq$ 200 g carbohydrate/d with adequate food energy for 3 d before testing.

After an overnight fast of 10–14 h, an indwelling catheter was inserted into an antecubital vein and was kept patent with a slow infusion of 0.45% saline. Test meals were given at 0800 and consisted of, in random order, a meal of 50 g glucose, 50 g protein, or a combination of 50 g glucose and 10, 30, or 50 g protein. Glucose (100 g/180 mL) was given as a standard glucose solution (Glutol, Paddock Laboratories, Minneapolis). The protein was given in the form of cooked, lean hamburger (6.5% fat). After potassium hydroxide hydrolysis the protein content was determined by the method of Zak and Cohen (10). The fat content was determined gravimetrically by ether extraction. The meal was browned in a nonstick frying pan and placed in a refrigerator until served. Cooking was completed by placing the meat in a microwave oven for 30 s. Beef protein was selected because it is a commonly ingested form of meat protein in our society. In addition, we had used it previously in our study of the dose response to protein in diabetic subjects. The beef was very low in fat and was considered to be less palatable than most meats eaten by Americans; however, it was not considered offensive to the volunteers.
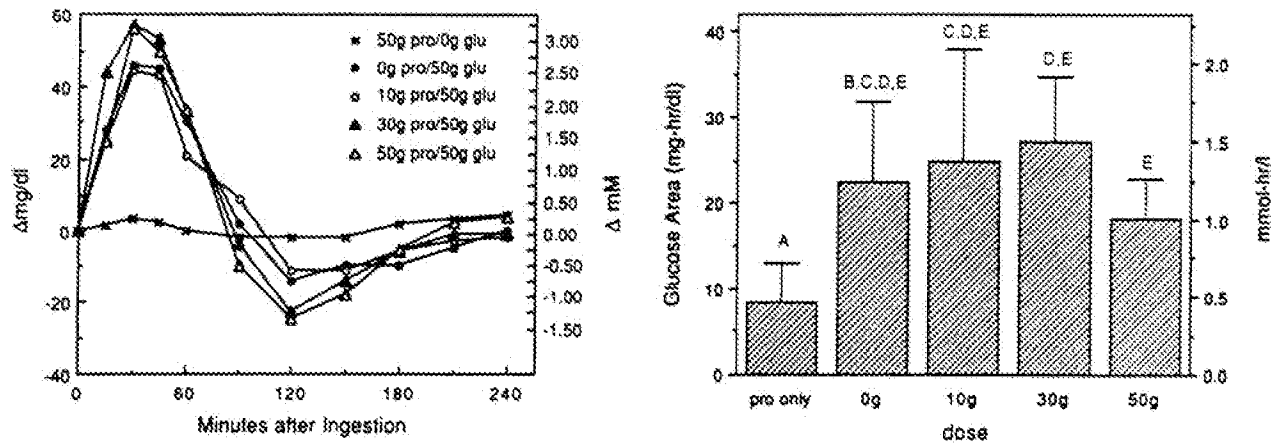
FIG 1. Left panel, glucose response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on glucose area. The incremental change in plasma glucose was determined for 4 h after ingestion of the meals ($n = 7$). The 0–50-g doses of protein were ingested together with 50 g glucose. Bar graphs indicate mean ± SEM. Areas are significantly different ($p < 0.05$) if they do not share a common superscript letter.

Blood samples were drawn before and at 15-min intervals for 1 h and then at 30-min intervals for 3 h after ingestion of the meal. Plasma glucose was determined by a glucose oxidase method with a glucose analyzer with an oxygen electrode (Beckman Instruments, Inc, Fullerton, CA). Serum immunoreactive insulin was measured by a standard double-antibody radioimmunoassay (RIA) method with kits produced by Endotech, Inc, Louisville. Glucagon was determined by RIA by use of 30 K antiserum purchased from Health Science Center (Dallas), and α-amino nitrogen was determined by the method of Goodwin (11). C peptide was measured by a double-antibody RIA method (12) with kits produced by Immuno-Nuclear Corp, Stillwater, MN.

The areas above the fasting baseline were calculated by using the trapezoid rule (13). The concentration at time zero was used as a constant baseline. In calculating the postmeal areas we assumed that the fasting concentration remained unchanged. Areas below the baseline were subtracted from areas above the baseline to give a net area. The analysis of variance (ANOVA) test with least significant difference was used to assess statistical significance (*Statview 512*, Apple Computer

Co). A p value of < 0.05 was the criterion for significance. Data are presented as the mean ± SEM.

Results

The mean baseline value of plasma glucose was 4.8 ± 0.1 mmol/L. It reached a peak 30 min after the ingestion of all meals containing glucose, regardless of the presence or absence of protein. It returned to near baseline concentrations by 90 min, decreased to a nadir at 120 min, and then reapproached baseline by 240 min (Fig 1, left panel). The ingestion of various amounts of protein with glucose did not significantly alter the curve seen with glucose alone. There was little change in plasma glucose concentration after ingestion of 50 g protein alone. The calculated net area under the curve for the plasma glucose concentration increased only slightly when protein was ingested alone (Fig 1, right panel). The plasma-glucose-area response to ingestion of glucose was much greater, as expected, but was not significantly affected by the simultaneous ingestion of various amounts of protein.
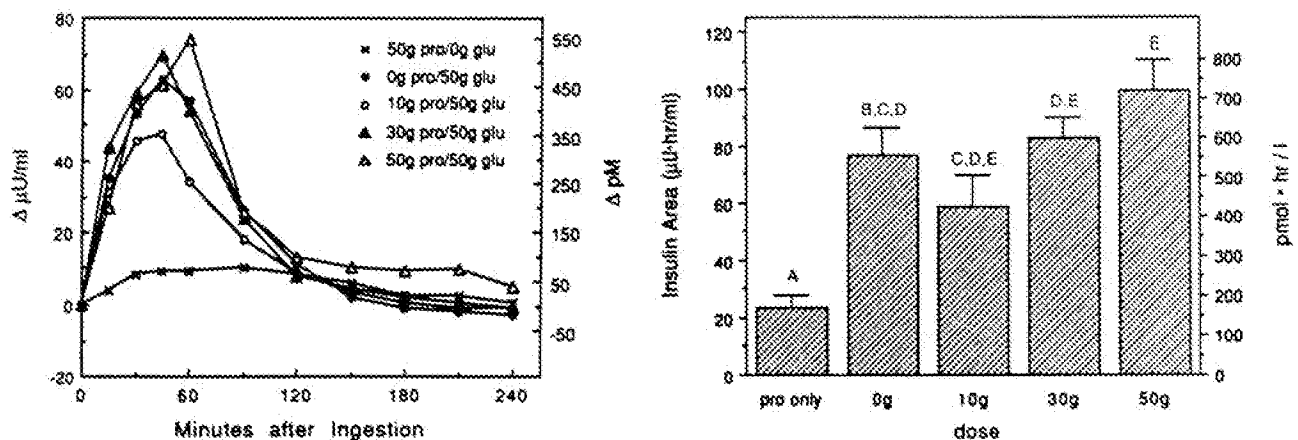

FIG 2. Left panel, insulin response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on insulin area. The incremental change in plasma insulin was determined as indicated in the legend for Figure 1.
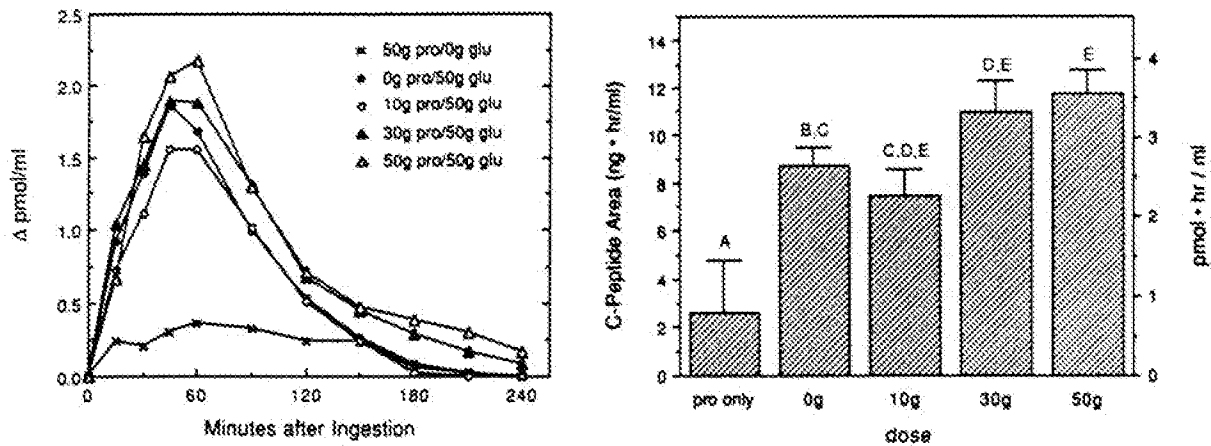
FIG 3. Left panel. C peptide response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on C peptide area. The incremental change in plasma C peptide was determined as indicated in the legend for Figure 1.

The initial mean insulin concentration was 96 ± 7 pmol/L. The peak occurred 45 min after ingestion of 50 g glucose and then returned to baseline at 180 min (Fig 2, left panel). When glucose and protein were ingested together, the peak insulin occurred at 45 min for all but the meal containing 50 g protein plus 50 g glucose. Peak insulin occurred 60 min after ingestion of the meal containing 50 g protein plus 50 g glucose. Insulin concentrations decreased to the baseline by 240 min after each of the meals. When 50 g protein was ingested alone, the insulin increase was quite modest but it remained elevated for 240 min (Fig 2, left panel). Addition of increasing amounts of protein to the meal containing 50 g glucose did not result in an increased insulin-area response until 50 g protein was given (Fig 2, right panel). As indicated previously, the sum of the mean insulin areas for the meals containing either 50 g protein only and 50 g glucose only was 100% that of 50 g protein and 50 g glucose together. Thus, these insulin responses were additive.

The mean baseline concentration of C peptide was 0.69 ± .06 pmol/mL. Like insulin, the C peptide concentration increased very modestly after the protein-only meal and increased to a peak at 45 min for all meals with glucose except for the meal containing glucose plus 50 g protein. After this meal, it peaked

15 min later (Fig 3, left panel). The curves were similar to those for plasma insulin. The C peptide area was not increased when 10 g protein was ingested with 50 g glucose compared with glucose alone. It was increased when 30 and 50 g protein were added (Fig 3, right panel).

The mean initial concentration of α-amino nitrogen was 3.1 ± 0.1 mmol/L. This rapidly decreased over the first 90 min after ingestion of 50 g glucose alone and remained near this nadir throughout the study period (Fig 4, left panel). After the meal of protein alone, there was a prompt increase in α-amino nitrogen over the first 90 min. This was then sustained throughout the study. After the glucose-plus-protein meals, the α-amino nitrogen concentrations initially remained near the baseline, then rapidly increased to a maximum at 150–180 min, except for the 10-g protein dose. The α-amino nitrogen concentration for this dose remained at the baseline. For the higher protein doses of 30 and 50 g, ingested with glucose, this increase remained for the 240 min of study. After glucose alone the area was negative. It increased progressively with increasing amounts of protein in the meal. The area response to 50 g protein alone was similar to that with 50 g protein plus 50 g glucose (Fig 4, right panel).
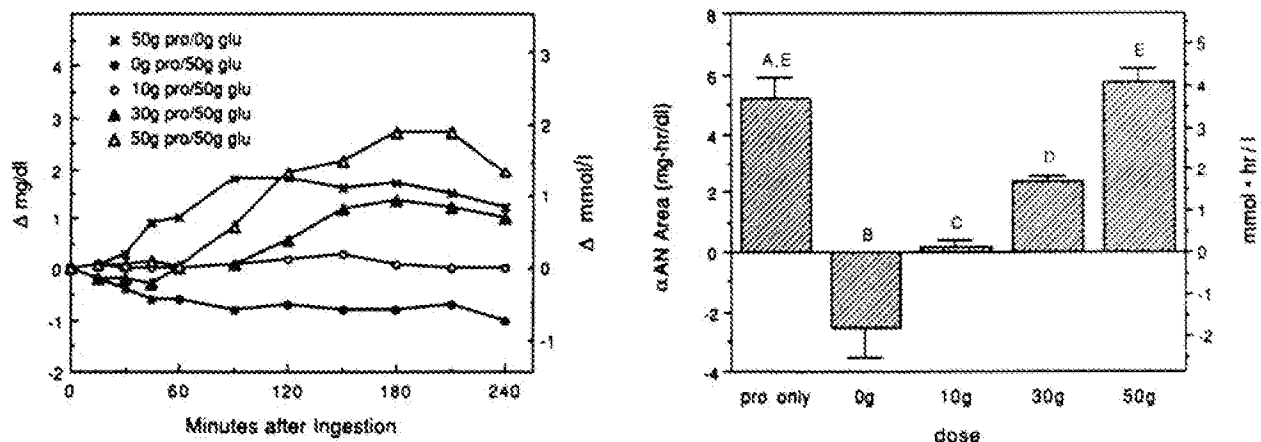

FIG 4. Left panel, α-amino nitrogen response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on α-amino nitrogen area. The incremental change in plasma α-amino nitrogen was determined as indicated in the legend for Figure 1.
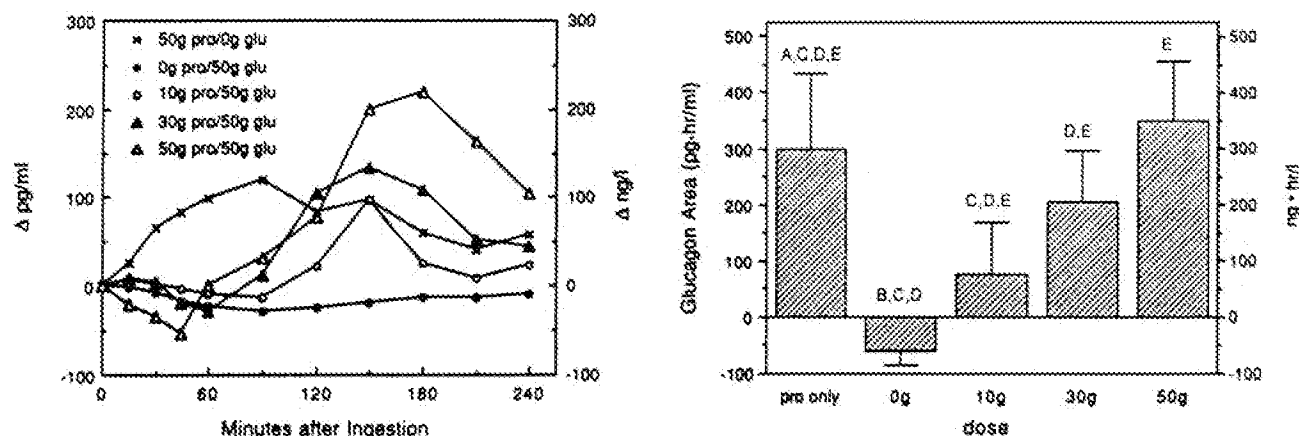
FIG 5. Left panel, glucagon response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on glucagon area. The incremental change in plasma glucagon was determined as indicated in the legend for Figure 1.

The mean initial value of glucagon was 211 ± 66 ng/L. After the ingestion of glucose alone, glucagon decreased modestly to a nadir at 90 min and then slowly returned to baseline. After the ingestion of protein alone there was a considerable increase, with a peak at 90 min. It then remained elevated for the 240 min of study (Fig 5, left panel). After meals containing glucose with 10 or 30 g protein, the mean glucagon concentration remained initially unchanged. It decreased modestly when 50 g protein was ingested with the glucose. Later in the time course, the concentration increased after meals containing protein. The rapidity of the onset and the magnitude of the rise correlated directly with the amount of protein ingested. In general, the curves were similar to those for α-amino nitrogen.

The area under the glucagon curve was negative after ingestion of the meal containing 50 g glucose (Fig 5, right panel). After all meals with protein, the area under the curve was positive and it increased progressively with increasing amounts of protein. The area under the curve resulting from ingestion of 50 g protein with 50 g glucose was not different from that observed when 50 g protein was ingested alone.

After the ingestion of all meals, nonesterified free fatty acids decreased from a mean baseline of 319 ± 50 mmol/L to reach

a plateau at ~60 min (Fig 6, left panel). The nadir reached was lower for all meals containing glucose compared with that resulting from ingestion of protein alone. Although the nadir reached was similar for all glucose-containing meals, the duration of time over which the free fatty acids remained depressed varied inversely with the amount of protein in the meal. Mean areas under the curve of nonesterified free fatty acids were not statistically different from protein alone, except for the meal containing glucose plus 30 g protein (Fig 6, right panel).

Discussion

When protein is added to a carbohydrate meal, a significant attenuation in the plasma glucose rise has been reported by some investigators (6, 7, 14, 15) but not by others (16, 17). In these studies, subjects with NIDDM (14, 17) or normal subjects were used (15, 16).

Spiller et al (15) and Day et al (16) studied the effect of various amounts of protein on metabolic response in nondiabetic subjects. Spiller et al (15) used breakfasts containing 0–49 g protein together with 58 g carbohydrate as a mixture of malto-
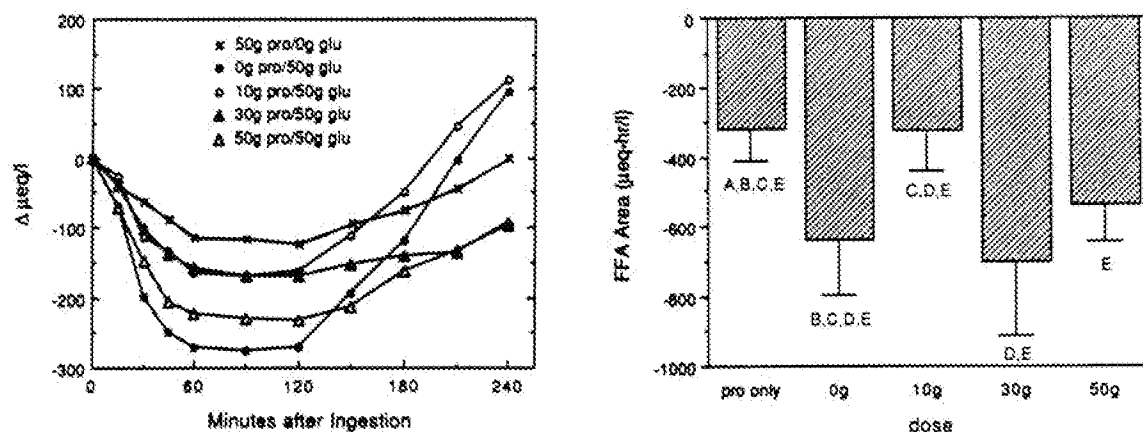

FIG 6. Left panel, nonesterified fatty acid response to ingestion of glucose, protein, or glucose plus protein. Right panel, effect of protein dose on nonesterified fatty acid area. The incremental change in plasma nonesterified fatty acid was determined as indicated in the legend for Figure 1. (1 μeq = 1 μmol.)
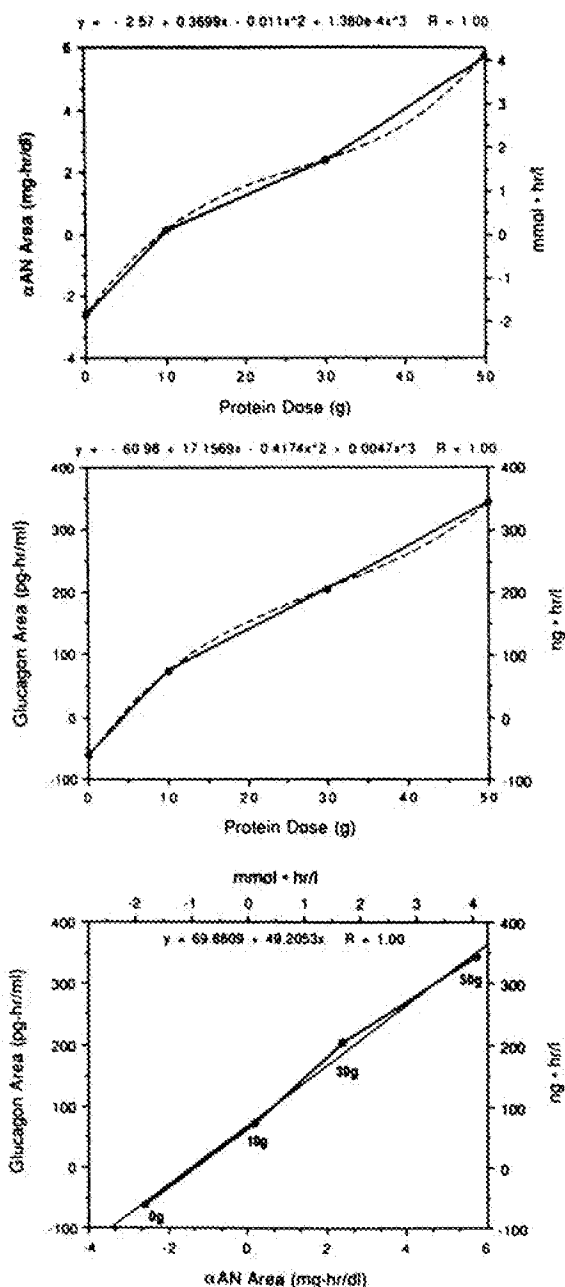
FIG 7. Top and middle panels, effect of protein dose on α-amino nitrogen area and glucagon area. Each protein dose was given with 50 g glucose. The curve was generated by computer, as was the equation for the curve. The best-fitting curve for the data of each graph was a third-order polynomial, indicating a nonlinear relationship between the dose of the protein ingested and α-amino nitrogen or glucagon area. Bottom panel, glucagon area vs α-amino nitrogen area. Each protein dose was given with 50 g glucose. The curve was generated by computer, as was the equation for the curve. The best-fitting curve for the data was first order, indicating a linear relation between the glucagon area and α-amino nitrogen area for the protein doses.

dextrins, fructose, and lactose. The area under the curve, determined by use of the trapezoid rule, with fasting glucose as a baseline, was measured for 2 h after the meal. A significant relationship was found between glucose area under the curve and the amount of protein in the meal. That is, the largest area under the glucose curve occurred without protein present, and the

area under the curve progressively decreased with an increased amount of protein in the meal. In this study we found no effect on the net glucose area integrated either over 4 h (Fig 1, right panel) or when integrated over 2 h (data not shown). The different results of the two studies may be due to the type and amount of carbohydrate ingested; however, this remains to be determined.

In the study by Spiller et al (15) the smallest amount of protein given (16 g) resulted in a significant increase in insulin-area response when integrated over a 2-h period. With ingestion of larger amounts of protein, the insulin-area response was not further increased. However, as pointed out by the authors, the insulin values at 2 h had not yet returned to baseline. Thus, the results could be misleading. Again, these results are considerably different from those we observed. In our study an increase in insulin-area response was only present with the 50-g dose and the C peptide area was only increased with a dose of ≥ 30 g.

The study protocol used by Day et al (16) differed in several ways from that used by Spiller et al (15) and ours, making comparisons difficult. The test meal was given at noon and the responses were measured for just 90 min. The meals contained various amounts of fat as well as a constant amount of carbohydrate and different amounts of protein. The amount of carbohydrate also was less (25 g). The protein content ranged from 3.6 to 75 g, and the source of the protein was not constant. In agreement with our results, protein addition did not have a significant effect on the glucose rise. When protein was present in amounts > 8 g, there was an increase in mean insulin area but the increase was not greater with ingestion of larger amounts of protein.

In a previous study of untreated NIDDM subjects that used the same meal protocol used in this study, we found that the insulin-area response increased linearly with respect to the quantity of protein ingested (7). The net glucose area also decreased in a concentration-dependent fashion with increasing doses of 10, 30, and 50 g protein. Thus, it is clear that the glucose and insulin responses to progressively larger amounts of protein given with a constant amount of glucose is different in normal and NIDDM subjects. The insulin secretory response is much more sensitive to protein ingestion in persons with NIDDM. The type of protein ingested also has effects on the insulin area response in NIDDM (18) and normal subjects (unpublished observations).

As we (8) reported previously, protein ingested alone led to a rapid rise in α-amino nitrogen concentration whereas glucose ingestion resulted in a rapid fall in concentration. Also, when 50 g protein and 50 g glucose were ingested together, there was little or no rise in α-amino nitrogen for the first 60 min (8). In the present study, a delay in the increase in α-amino nitrogen concentration also was present when 30 g protein was ingested with the glucose but the duration of this delay was shorter. With 10 g protein there was little change in α-amino nitrogen.

It was reported that hyperglycemia induced in normal subjects significantly slows gastric emptying (19, 20). Therefore, it is possible that the rise of plasma glucose, resulting from the glucose in the meal, delayed emptying of protein into the duodenum and thus delayed its digestion. However, because the area under the curve was negative after the glucose meal but remained near the baseline for ≥ 60 min when protein was ingested with the glucose, it is possible that the unchanged α-amino nitrogen concentration over this time resulted from a

rise in insulin that was just balanced by the influx of amino acid from the gut. This is unlikely because the results were the same regardless of the protein dose. Insulin-dependent suppression of amino acid entry into the plasma from muscle is well documented (21, 22). When the α-amino-nitrogen-area response was integrated over 4 h, there was a curvilinear relationship between the amount of protein ingested and the area response (Fig 7, top panel). This was best defined by a third-degree polynomial expression. From these data it appears that the null point (ie, the protein dose) at which there is essentially no net change in area response would occur with a protein dose of 9 g, that is, a glucose-to-protein ratio of ~5:1.

Our results are in agreement with previous studies indicating that protein ingestion stimulates while glucose ingestion suppresses glucagon secretion (23–28). In addition, our data confirm the observation that the circulating glucagon concentration depends on the ratio of protein to carbohydrate in the meal (16, 25). When glucose was present in the meal, a significant rise in glucagon concentration did not occur for ≥ 60 min, regardless of the protein dose. This was the time at which the α-amino nitrogen concentration began to rise. As with the α-amino nitrogen concentration, the glucagon-area response integrated over 4 h showed a sigmoidal relationship with the amount of protein ingested (Fig 7, middle panel).

The importance of a rise in circulating amino acids and/or the digestion of protein on the glucagon secretion is shown by the close correlation between the α-amino nitrogen and glucagon (Fig 7, bottom panel). This linear relationship indicates that a particular α-amino nitrogen response would be associated with a predictable glucagon response. For example, a rise in α-amino nitrogen of 0.7 mmol·h·L^{-1} would correspond to a rise of glucagon of ~50 ng·h·L^{-1}.

The amount of protein ingested with 50 g glucose that resulted in no net change in glucagon area was calculated to be only 5 g. Thus, it is clear that ingested protein is much more potent in stimulating a rise in glucagon concentration than glucose is in suppressing it. Our data suggest that the effect of beef protein is 10-fold greater than that of glucose on a weight basis. To our knowledge the sensitivity of glucagon to the ingestion of protein has not been determined. ■

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